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1. Your reference — P031378GB

2. Patent application number *(The Patent Office will fill in this part)* — 0218040.4

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P01/7700 0.00-0218040.4

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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UNITED KINGDOM

Patents ADP number *(if you know it)* 8258675001

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention — CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)* — Carpmaels & Ransford

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

43 Bloomsbury Square
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Patents ADP number *(if you know it)* — 83001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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Request for substantive examination *(Patents Form 10/77)*	
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11. I/We request the grant of a patent on the basis of this application.

Signature: Carpmaels + Ransford Date: 2nd August 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Carpmaels & Ransford

Dr. Bruce R. Cockerton **020-7242 8692**

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DUPLICATE

CHEMICAL COMPOUNDS

This invention relates to substituted thienyl-hydroxamic acids, their preparation and pharmaceutical compositions containing these compounds for treating diseases associated with histone deacetylase enzymatic activity.

In eukaryotic cells, DNA is tightly associated with histones to form a compact complex called chromatin. The histones, generally highly conserved across eukaryotic species, constitute a family of proteins which are rich in basic amino acids that contact the phosphate groups of DNA.

There are five main classes of histones, H1, H2A, H2B, H3 and H4. Four pairs of each of H2A, H2B, H3 and H4 together form a disk-shaped octomeric protein core, around which DNA is wound (with the basic amino acids of the histones interacting with the negatively charged phosphate groups of the DNA) to form a nucleosome. Approximately 146 base pairs of DNA wrap around a histone core to make up a nucleosome particle, the repeating structural motif of chromatin.

Histone deacetylases (HDACs) are part of transcriptional corepressor complexes and play key roles in regulating chromatin structure. Three different classes of human HDACs have been defined based on their homology to HDACs found in *Saccharomyces cerevisiae*. Class I HDACs (HDAC1, 2, 3, and 8) are derived from the yeast transcriptional regulator RPD3. Class II HDACs (HDAC4, 5, 6, 7, 9, and 10) are similar to HDA1, another deacetylase in yeast. Class III HDACs are related to the yeast silencing protein SIR2 and are dependent on NAD for enzymatic activity.

Reversible acetylation of histones is a major regulator of gene expression that acts by altering accessibility of transcription factors to DNA. In normal cells, histone deacetylase (HDA) and histone acetyltransferases (HAT) together control the level of acetylation of histones to maintain a balance. Histone acetylation has a key role in transcriptional activation, whereas deacetylation of histones correlates with the transcriptional repression and silencing of genes [for a review of histone deacetylation see Kouzarides *Curr. Opin. Genet. Dev.*, 9:40-48 (1999); and Pazin and Kadonaga, *Cell*, 89:325-8 (1997)]. Genetic

repression may have an important role in neuronal ageing, atrophy and degenerative diseases.

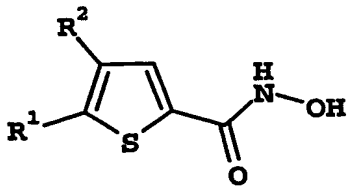
Moreover, histone deacetylases have been shown to regulate the activity of non-histone proteins through the modification of their acetylation level. These include steroid receptors such as estrogen and androgen receptors [Wang *et al*, *J. Biol. Chem.*, 276:18375-83 (2001), Gaughan *et al*, *J. Biol. Chem.*, 277: 25904-13 (2002)], transcription factors such as p53, E2F and myoD [Luo *et al*, *Nature*, 408:377-381 (2000); Ito *et al*, *EMBO J*, 19:1176-1179 (2001); Sartorelli *et al*, *Mol. Cell*, 4:725-734 (1999)], and cytoplasmic proteins such as α-tubulin [Hubbert *et al*, *Nature*, 417:455-458 (2002)].

There are currently several known inhibitors, both natural and synthetic, of HDAC. Some natural inhibitors include: (i) trapoxin B; (ii) trichostatin A [Richon *et al.*, *Proc. Natl. Acad. Sci. USA*, 95: 3003-3007 (1998)]; and (iii) chlamydocin. Synthetic inhibitors include suberoyl anilide hydroxamic acid [Yoshida and Beppu, *Exper. Cell Res.*,177:122-131 (1988)] and phenylbutyrate.

Trichostatin A has been shown to cause arrest of rat fibroblasts at both G_1 and G_2 phases of the cell cycle, implicating HDAC in cell cycle regulation [Yoshida and Beppu, *Exper. Cell Res.*, 177:122-131 (1988)]. Trichostatin A and suberoyl anilide hydroxamic acid have been shown to inhibit cell growth, induce terminal differentiation and prevent the formation of tumors in mice [Finnin *et al*, *Nature*, 401: 188-193 (1999)]. Trapoxin, trichostatin, and depudecin have been used to study gene regulation by HDAC-mediated chromatin remodeling [Christian A. Hassig, Stuart L. Schreiber, *Curr. Opinion in Chem. Biol.*, 1997, 1, 300-308; Christian A. Hassig, Jeffrey K. Tong, Stuart L. Schreiber, *Chem. & Biol.*, 1997, 4, 783-789; Christian A. Hassig, Jeffrey K. Tong, Tracey C. Fleischer, Takashi Owa, Phyllis Grable, Donald E. Ayer, Stuart L. Schreiber, *Proc. Natl. Acad. Sci., U.S.A.*, 1998, 95, 3519-3524; Ho Jeong Kwon, Takashi Owa, Christian A. Hassig, Junichi Shimada, Stuart L. Schreiber, *Proc. Natl. Acad. Sci., U.S.A.* 1998, 95, 3356-3361].

It is an object of the present invention to provide inhibitors of histone deacetylase.

Thus, in one aspect, the present invention provides compounds of formula (I):



(I)

in which

R^1 represents aryl or heteroaryl, each optionally substituted by one or more groups selected from R^3, alkylenedioxy, carboxy, cyano, halo, hydroxy, nitro, haloalkyl, haloalkoxy, $-C(=O)-R^3$, $-C(=O)-OR^3$, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=O)-OR^3$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-C(=Z)-R^3$, $-O-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^3$, $-OR^3$, $-O-C(=O)R^3$, -SH, $-SR^3$, $-SOR^3$, $-SO_2R^3$ and $-SO_2-NR^4R^5$;

R^2 represents hydrogen, chloro, cyano, fluoro, alkoxy, alkyl, or haloalkyl;

R^3 represents aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl or R^m;

R^4 and R^5 independently represent a group selected from hydrogen, alkyl, alkenyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl, wherein said alkyl or alkenyl are optionally substituted by aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl; or the group $-NR^4R^5$ may form a cyclic amine;

R^6 represents hydrogen or lower alkyl;

R^m represents alkyl, alkenyl and alkynyl, wherein said alkyl, alkenyl or alkynyl are optionally substituted by one or more groups selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, hydroxy, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=Z)-R^n$,

$-O-C(=O)-NR^4R^5$, $-NR^6-C(=O)-OR^n$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^n$, $-OR^n$, $-SOR^n$, SO_2R^n and $-SO_2-NR^4R^5$;

R^n represents alkyl, alkenyl or alkynyl, optionally substituted by one or more groups selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, hydroxy and halogen; or R^n represents aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl; and

Z is O or S,

and corresponding *N*-oxides, pharmaceutically acceptable salts, solvates and prodrugs of such compounds.

A second aspect of the invention is a pharmaceutical composition comprising a compound of Formula I or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof, in admixture with a pharmaceutically acceptable carrier or excipient.

A third aspect of the invention is a compound of Formula I or *an N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof for use in therapy.

A fourth aspect of the invention is the use of a compound of Formula I, or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof, in the manufacture of a medicament for the treatment of a disease in which inhibition of histone deacetylase can prevent, inhibit or ameliorate the pathology and/or symptomatology of the disease.

A fifth aspect of the invention is a method for treating a disease in a patient in which inhibition of histone deacetylase can prevent, inhibit or ameliorate the pathology and/or symptomatology of the disease, which method comprises administering to the patient a therapeutically effective amount of compound of Formula I or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof.

A sixth aspect of the invention is a method of inhibiting histone deacetylase in a cell, comprising contacting a cell in which inhibition of histone deacetylase is desired with a compound of Formula I or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof.

A seventh aspect of the invention is a method of preparing a compound of formula I or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof.

An eighth aspect of the invention is a method of making a pharmaceutical composition comprising combining a compound of formula (I), or an *N*-oxide, pharmaceutically acceptable salt, solvate or prodrug thereof, with a pharmaceutically acceptable carrier or excipient.

For purposes of the present invention, the following definitions as used throughout the description of the invention shall be understood to have the following meanings:

"Compounds of the invention", and equivalent expressions, are meant to embrace compounds of general formula (I) as hereinbefore described, their *N*-oxides, their prodrugs, their pharmaceutically acceptable salts and their solvates, where the context so permits.

"Histone deacetylase" and "HDAC" are intended to refer to any one of a family of enzymes that remove acetyl groups from lysine residues of proteins including, but not limited to, histones, transcription factors, steroid receptors and tubulin. Unless otherwise indicated the term histone is meant to refer to any histone protein, including H1, H2A, H2B, H3, H4 and H5 from any species. In one preferred embodiment the histone deacetylase is a human HDAC, including, but not limited to, HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, HDAC-8, HDAC-9, and HDAC-10. In another preferred embodiment the histone deacetylase is derived from a protozoal or fungal source.

"Patient" includes both human and other mammals.

For purposes of the present invention, the following chemical terms as used above, and throughout the description of the invention, and unless otherwise indicated, shall be understood to have the following meanings:

"Acyl" means an alkyl-CO- group in which the alkyl group is as described herein.

"Alkenyl" as a group or part of a group denotes an aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched having from 2 to 12 carbon atoms, preferably 2-6 carbon atoms, in the chain. Exemplary alkenyl groups include ethenyl, and propenyl.

"Alkoxy" means an -O-alkyl group in which alkyl is as defined below. Exemplary alkoxy groups include methoxy and ethoxy.

"Alkoxycarbonyl" means an -C(=O)-O-alkyl group in which alkyl is as defined below. Exemplary alkoxycarbonyl groups include methoxycarbonyl and ethoxycarbonyl.

"Alkyl" as a group or part of a group refers to a straight or branched chain saturated aliphatic hydrocarbon group having from 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms, in the chain. Exemplary alkyl groups include methyl, ethyl, 1-propyl, and 2-propyl.

"Alkylamino" means a -NH-alkyl group in which alkyl is as defined above. Exemplary alkylamino groups include methylamino and ethylamino.

"Alkylene" means $-(CH_2)_n-$, where n may be 1 to 3.

"Alkylenedioxy" means a -O-alkylene-O- group in which alkylene is as defined above. Exemplary alkylenedioxy groups include methylenedioxy and ethylenedioxy.

"Alkylsufinyl" means a -SO-alkyl group in which alkyl is as defined above. Exemplary alkylsulfinyl groups include methylsulfinyl and ethylsulfinyl.

"Alkylsufonyl" means a -SO_2-alkyl group in which alkyl is as defined above. Exemplary alkylsulfonyl groups include methylsulfonyl and ethylsulfonyl.

"Alkylthio" means a -S-alkyl group in which alkyl is as defined above. Exemplary alkylthio groups include methylthio and ethylthio.

"Alkynyl" as a group or part of a group means an aliphatic hydrocarbon group containing a carbon-carbon triple bond and which may be straight or branched having from 2 to 6 carbon atoms in the chain. Exemplary alkynyl groups include ethynyl and propynyl.

"Aryl" as a group or part of a group denotes: (i) an optionally substituted monocyclic or multicyclic aromatic carbocyclic moiety of from 6 to 14 carbon atoms, preferably from 6 to 10 carbon atoms, such as phenyl or naphthyl, and in one embodiment preferably phenyl; or (ii) an optionally substituted partially saturated bicyclic aromatic carbocyclic moiety in which a phenyl and a C_{5-7} cycloalkyl or C_{5-7} cycloalkenyl group are fused together to form a cyclic structure, such as tetrahydronaphthyl, indenyl or indanyl. The aryl group may be substituted by one or more substituent groups.

"Arylalkenyl" means an aryl-alkenyl- group in which the aryl and alkenyl are as previously described. Exemplary arylalkenyl groups include styryl and phenylallyl.

"Arylalkyl" means an aryl-alkyl- group in which the aryl and alkyl moieties are as previously described. Preferred arylalkyl groups contain a C_{1-4} alkyl moiety. Exemplary arylalkyl groups include benzyl, phenethyl and naphthlenemethyl.

"Arylalkynyl" means an aryl-alkynyl- group in which the aryl and alkynyl are as previously described. Exemplary arylalkynyl groups include phenylethynyl.

"Cyclic amine" means an optionally substituted 3 to 8 membered monocyclic cycloalkyl ring system where one of the ring carbon atoms is replaced by nitrogen and which (i) may optionally contain an additional heteroatom selected from O, S or NR (where R is hydrogen, alkyl, arylalkyl, and aryl) and (ii) may be fused to additional aryl or heteroaryl ring to form a bicyclic ring system. Exemplary cyclic amines include pyrrolidine,

piperidine, morpholine, piperazine, indoline. The cyclic amine group may be substituted by one or more substituent groups.

"Cycloalkenyl" means an optionally substituted non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and having from 5 to 10 carbon atoms. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl or cycloheptenyl. The cycloalkenyl group may be substituted by one or more substituent groups.

"Cycloalkenylalkyl" means a cycloalkenyl-alkyl- group in which the cycloalkenyl and alkyl moieties are as previously described. Exemplary cycloalkenylalkyl groups include cyclopentenylmethyl, cyclohexenylmethyl or cycloheptenylmethyl.

"Cycloalkyl" means an optionally substituted saturated monocyclic or bicyclic ring system of from 3 to 12 carbon atoms, preferably from 3 to 8 carbon atoms, and more preferably from 3 to 6 carbon atoms. Exemplary monocyclic cycloalkyl rings include cyclopropyl, cyclopentyl, cyclohexyl and cycloheptyl. The cycloalkyl group may be substituted by one or more substituent groups.

"Cycloalkylalkyl" means a cycloalkyl-alkyl- group in which the cycloalkyl and alkyl moieties are as previously described. Exemplary monocyclic cycloalkylalkyl groups include cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl and cycloheptylmethyl.

"Dialkylamino" means a $-N(alkyl)_2$ group in which alkyl is as defined above. Exemplary dialkylamino groups include dimethylamino and diethylamino.

"Halo" or "halogen" means fluoro, chloro, bromo, or iodo. Preferred are fluoro or chloro.

"Haloalkoxy" means an -O-alkyl group in which the alkyl is substituted by one or more halo atoms. Exemplary haloalkyl groups include trifluoromethoxy and difluoromethoxy.

"Haloalkyl" means an alkyl group which is substituted by one or more halo atoms. Exemplary haloalkyl groups include trifluoromethyl.

"Heteroaryl" as a group or part of a group denotes: (i) an optionally substituted aromatic monocyclic or multicyclic organic moiety of from 5 to 14 ring atoms, preferably from 5 to 10 ring atoms, in which one or more of the ring atoms is/are element(s) other than carbon, for example nitrogen, oxygen or sulfur (examples of such groups include benzimidazolyl, benzoxazolyl, benzthiazolyl, benzofuranyl, benzothienyl, furyl, imidazolyl, indolyl, indolizinyl, isoxazolyl, isoquinolinyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrazinyl, pyridazinyl, pyrazolyl, pyridyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, tetrazolyl, 1,3,4-thiadiazolyl, thiazolyl, thienyl and triazolyl groups; (ii) an optionally substituted partially saturated multicyclic heterocarbocyclic moiety in which a heteroaryl and a cycloalkyl or cycloalkenyl group are fused together to form a cyclic structure (examples of such groups include pyrindanyl groups). The heteroaryl group may be substituted by one or more substituent groups.

"Heteroarylalkenyl" means a heteroaryl-alkenyl- group in which the heteroaryl and alkenyl moieties are as previously described. Exemplary heteroarylalkenyl groups include pyridylethenyl and pyridylallyl.

"Heteroarylalkyl" means a heteroaryl-alkyl- group in which the heteroaryl and alkyl moieties are as previously described. Preferred heteroarylalkyl groups contain a lower alkyl moiety. Exemplary heteroarylalkyl groups include pyridylmethyl.

"Heteroarylalkynyl" means a heteroaryl-alkynyl- group in which the heteroaryl and alkynyl moieties are as previously described. Exemplary heteroarylalkenyl groups include pyridylethynyl.

"Heterocycloalkyl" means: (i) an optionally substituted cycloalkyl group of from 4 to 8 ring members which contains one or more heteroatoms selected from O, S or NR; (ii) an optionally substituted partially saturated multicyclic heterocarbocyclic moiety in which an aryl (or heteroaryl ring) and a heterocycloalkyl group are fused together to form a cyclic structure (examples of such groups include dihydrobenzofuranyl, indolinyl and tetrahydroquinolinyl groups); (iii) a cycloalkyl group of from 4 to 8 ring members which contains C(=O)NR and C(=O)NRC(=O). (examples of such groups include succinimidyl

and 2-oxopyrrolidinyl). The heterocycloalkyl group may be substituted by one or more substituent groups.

"Heterocycloalkylalkyl" means a heterocycloalkyl-alkyl- group in which the heterocycloalkyl and alkyl moieties are as previously described.

"Lower alkyl" as a group means unless otherwise specified, an aliphatic hydrocarbon group which may be straight or branched having 1 to 4 carbon atoms in the chain, i.e. methyl, ethyl, propyl (n-propyl or isopropyl) or butyl (n-butyl, isobutyl or tertiary-butyl).

"Pharmaceutically acceptable salt" means a physiologically or toxicologically tolerable salt and include, when appropriate, pharmaceutically acceptable base addition salts and pharmaceutically acceptable acid addition salts. For example (i) where a compound of the invention contains one or more acidic groups, for example carboxy groups, pharmaceutically acceptable base addition salts that may be formed include sodium, potassium, calcium, magnesium and ammonium salts, or salts with organic amines, such as, diethylamine, N-methyl-glucamine, diethanolamine or amino acids (e.g. lysine) and the like; (ii) where a compound of the invention contains a basic group, such as an amino group, pharmaceutically acceptable acid addition salts that may be formed include hydrochlorides, hydrobromides, phosphates, acetates, citrates, lactates, tartrates, malonates, methanesulphonates and the like.

"Prodrug" means a compound which is convertible *in vivo* by metabolic means (e.g. by hydrolysis) to a compound of formula (I). For example an ester prodrug of a compound of formula (I) containing a hydroxy group may be convertible by hydrolysis *in vivo* to the parent molecule. Suitable esters of compounds of formula (I) containing a hydroxy group, are for example acetates, citrates, lactates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-β-hydroxynaphthoates, gentisates, isethionates, di-*p*-toluoyltartrates, methanesulphonates, ethanesulphonates, benzenesulphonates, p-toluenesulphonates, cyclohexylsulphamates and quinates. As another example an ester prodrug of a compound of formula (I) containing a carboxy group may be convertible by hydrolysis *in vivo* to the parent molecule (Examples of ester prodrugs are those described by F. J. Leinweber, Drug Metab. Res., 18:379 [1987]).

"Saturated" pertains to compounds and/or groups which do not have any carbon-carbon double bonds or carbon-carbon triple bonds.

The cyclic groups referred to above, namely, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl and cyclic amine may be substituted by one or more substituent groups. Suitable optional substituents include acyl (e.g. $-C(=O)CH_3$), alkoxy (e,g, $-OCH_3$), alkoxycarbonyl (e.g. $-C(=O)-OCH_3$), alkylamino (e.g. $-NHCH_3$), alkylenedioxy (e.g. $-O-CH_2-O-$), alkylsulfinyl (e.g. $-SOCH_3$), alkylsulfonyl (e.g. $-SO_2CH_3$), alkylthio (e.g. $-SCH_3$), amino, aminoalkyl (e.g. $-CH_2NH_2$), arylalkyl (e.g. $-CH_2Ph$ or $-CH_2-CH_2-Ph$), cyano, dialkylamino (e.g. $-N(CH_3)_2$), halo, haloalkoxy (e.g. $-OCF_3$ or $-OCHF_2$), haloalkyl (e.g. $-CF_3$), alkyl (e.g. $-CH_3$ or $-CH_2CH_3$), hydroxy, formyl and nitro.

Compounds of the invention may exist in one or more geometrical, optical, enantiomeric, diastereomeric and tautomeric forms, including but not limited to *cis*- and *trans*-forms, *E*- and *Z*-forms, *R*- *S*- and *meso*-forms, keto-, and enol-forms. Unless otherwise stated a reference to a particular compound includes all such isomeric forms, including racemic and other mixtures thereof. Where appropriate such isomers can be separated from their mixtures by the application or adaptation of known methods (e.g. chromatographic techniques and recrystallisation techniques). Where appropriate such isomers may be prepared by the application of adaptation of known methods (e.g. asymmetric synthesis).

With reference to formula (I) above, particular and preferred embodiments are described below.

Where R^1 is aryl or heteroaryl substituted by one or more haloalkyl groups, said haloalkyl group is preferably selected from trifluoromethyl. Where R^1 is aryl or heteroaryl substituted by one or more haloalkoxy groups, said haloalkoxy group is preferably selected from trifluoromethoxy or difluoromethoxy.

R^1 may particularly represent optionally substituted phenyl. Preferred groups for R^1 include phenyl or 4-methoxyphenyl.

R^1 may also particularly represent optionally substituted monocyclic heteroaryl, preferably optionally substituted imidazolyl, isoxazolyl, oxadiazolyl, pyrazolyl, pyridinyl, thienyl and pyrimidinyl, more preferably optionally substituted imidazolyl, pyrazolyl, pyridinyl and pyrimidinyl, particularly 2-imidazolyl, 3-pyrazolyl, 2-pyridinyl and 2-pyrimidinyl. Preferably, where R^1 is heteroaryl, it is preferably attached to the thienyl group of formula (I) above via a ring carbon atom of R^1, and in one embodiment via a ring carbon atom which is adjacent to a heteroatom. Preferred optional substituents include alkyl (preferably lower alkyl) and haloalkyl (preferably trifluoromethyl). Where the optional substituent is alkyl, the alkyl may be substituted, preferably by aryl or heteroaryl which in turn may be optionally substituted as described hereinabove. Particularly preferred substituents are arylalkyl, and heteroarylalkyl. R^1 especially represents 1-(2-phenylethyl)-1*H*-pyrazol-3-yl, 1-benzyl-1*H*-pyrazol-3-yl, 4-trifluoromethyl-1*H*-imidazol-2-yl, pyridin-2-yl, 5-trifluoromethyl-1*H*-pyrazol-3-yl, 1-methyl-1*H*-pyrazol-3-yl, 2-methyl-2*H*-pyrazol-3-yl, 1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl, 2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl, 1*H*-pyrazol-3-yl, pyridin-4-yl, 5-trifluoromethylisoxazol-3-yl, 3-methyl[1,2,4]oxadiazol-5-yl, or thiophene-2-yl.

R^2 may particularly represent hydrogen.

Where R^2 is alkyl, said alkyl group is preferably selected from lower alkyl, preferably methyl. Where R^2 is alkoxy, said alkoxy group is preferably selected from lower alkoxy, preferably methoxy. Where R^2 is haloalkyl, said haloalkyl group is preferably selected from trifluoromethyl.

In one embodiment, R^3 and R^n are independently selected from alkyl, alkenyl, alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroalkylalkenyl, heteroalkynyl, cycloalkylalkyl, cycloalkenylalkyl, heterocycloalkylalkyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl and heterocycloalkyl.

In one embodiment, R^3 and R^n are independently selected from alkyl, preferably lower alkyl, preferably methyl or ethyl.

In one embodiment, R^4 and R^5 are independently selected from hydrogen, alkyl, alkenyl, aryl, arylalkyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, heteroaryl, heterocycloalkyl or heteroarylalkyl; or the group $-NR^4R^5$ may form a cyclic amine;

In an alternative embodiment R^4 and R^5 are independently selected from hydrogen, alkyl, alkenyl, aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl or heteroarylalkyl; or the group $-NR^4R^5$ may form a cyclic amine;

In a further embodiment, R^4 and R^5 are independently selected from hydrogen and alkyl (preferably lower alkyl, preferably methyl).

In one embodiment, R^m is alkyl, alkenyl, alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroalkylalkenyl, heteroalkynyl, cycloalkylalkyl, cycloalkenylalkyl or heterocycloalkylalkyl.

In a preferred embodiment, R^1 is substituted by an alkyl, alkenyl or alkynyl group, preferably an alkyl or alkenyl group, optionally substituted by one or more groups selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, hydroxy, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=Z)-R^n$, $-O-C(=O)-NR^4R^5$, $-NR^6-C(=O)-OR^n$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^n$, $-OR^n$, $-SOR^n$, SO_2R^n and $-SO_2-NR^4R^5$. In a particularly preferred embodiment, said alkyl, alkenyl or alkynyl group is substituted by a group selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl and heterocycloalkyl, and optionally further substituted by a group selected from hydroxy, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=Z)-R^n$, $-O-C(=O)-NR^4R^5$, $-NR^6-C(=O)-OR^n$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^n$, $-OR^n$, $-SOR^n$, SO_2R^n and $-SO_2-NR^4R^5$. In a further particularly preferred embodiment, said alkyl, alkenyl or alkynyl group is substituted by a group selected from $-C(=Z)-NR^4R^5$, $-NR^6-C(=Z)-R^n$, $-O-C(=O)-NR^4R^5$, $-NR^6-C(=O)-OR^n$ and $-NR^6-C(=O)-NR^4R^5$, and in one embodiment from $-C(=Z)-NR^4R^5$ and $-NR^6-C(=Z)-R^n$, preferably wherein Z is O, wherein R^4, R^5 or R^n is a cyclic group as defined herein.

Particular compounds of the invention are:-

5-(2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-phenyl-thiophene-2-carboxylic acid hydroxyamide;

5-pyridin-2-yl-thiophene-2-carboxylic acid hydroxyamide;

[2,2']bithiophenyl-5-carboxylic acid hydroxyamide;

5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid hydroxyamide;

5-(2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid hydroxyamide;

and corresponding *N*-oxides, pharmaceutically acceptable salts, solvates and prodrugs of such compounds.

Preferred compounds of the invention are:

5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;

5-pyridin-2-yl-thiophene-2-carboxylic acid hydroxyamide;

and corresponding *N*-oxides, pharmaceutically acceptable salts, solvates and prodrugs of such compounds.

The present invention provides compounds that inhibit HDAC activity according to the tests described in the literature and in the Biological Activity section of this document. The therapeutic application of these compounds is pertinent to any disease that is known to be

at least in part mediated by HDAC activity or whose symptoms are known to be alleviated by HDAC inhibitors (such as Trichostatin-A, suberoyl anilide hydroxamic acid, Trapoxin and depudecin). For example, these compounds could be beneficial for the treatment of cancer, psoriasis, fibroproliferative disorders (e.g. liver fibrosis), smooth muscle cell proliferation disorders (e.g. arteriosclerosis, restenosis), inflammatory diseases and conditions treatable by immune modulation (e.g. rheumatoid arthritis, autoimmune diabetes, lupus, allergies), neurodegenerative disorders (e.g. Huntington's disease), diseases involving angiogenesis (e.g. cancer, psoriasis, rheumatoid arthritis, retinal diseases such as diabetic retinopathy, age-related macular degeneration, interstitial keratitis, rubeotic glaucoma), fungal and parasitic infections (e.g. malaria, protozoal infections) and haematopoietic disorders (e.g. anaemia, sickle cell anaemia, thalassemia) .

Thus, in one embodiment, the present invention is intended for the treatment of diseases caused by increased cell proliferation. These include, but are not limited to, primary and metastatic cancers of different origin (including those triggered by viral infections such as EBV, HIV, hepatitis B and C and KSHV), fibrosis of the liver, lung, kidney, heart and skin caused by myofibroblasts proliferation and increased production of extracellular matrix proteins [Niki *et al*, *Hepatology*, 29:858-67 (1999)], and inflammatory diseases.

In another embodiment, the invention is also aimed at the treatment of protozoal infections including, but not limited to, malaria, toxoplasmosis and coccidiosis.

In another embodiment, the invention is aimed at the treatment of diseases caused by expanded polyglutamine repeats resulting in histone hypoacetylation including, but not limited to, neurodegenerative disorders such as Huntington's disease.

The compounds of formula I may be used or administered in combination with one or more additional drug(s) and/or procedures (such as radiotherapy in the case of cancer) useful in the treatment of the disorders mentioned above, the components being in the same formulation or in separate formulations for administration simultaneously or sequentially. The additional drug(s) may or may not be HDAC inhbitors.

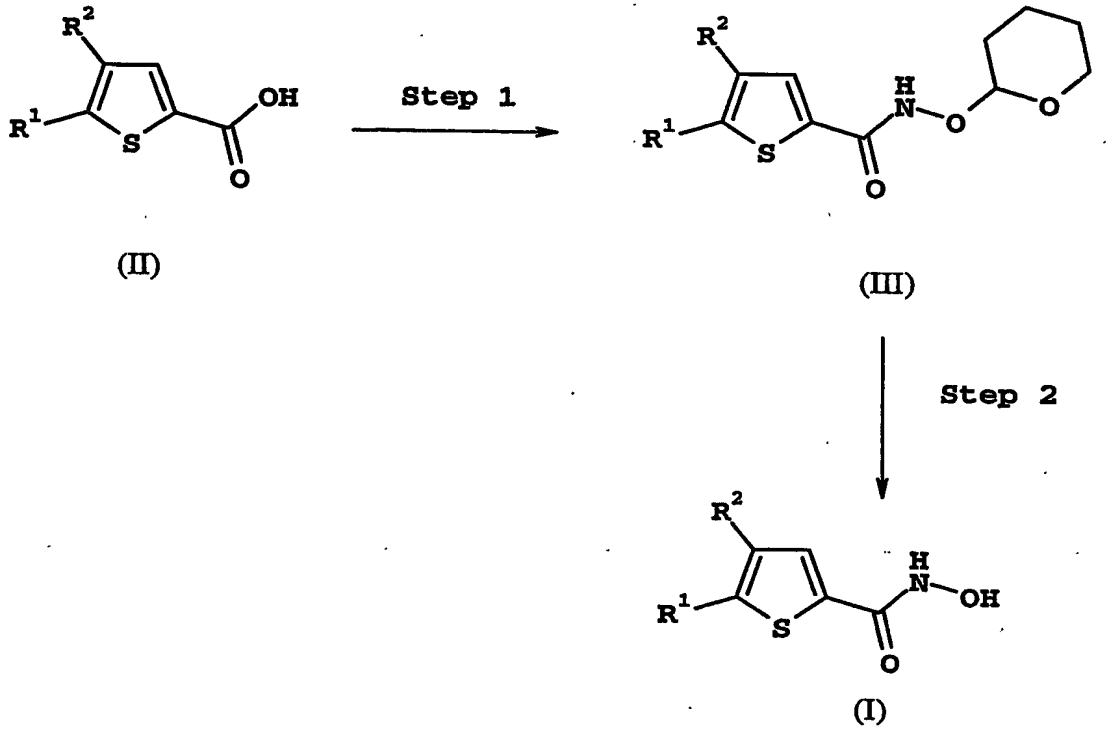
The thienyl-hydroxamic acids of the present invention may be prepared, for example, by the application or adaptation of methods described herein. They may also be prepared by known organic synthesis methods for example those described by R. C. Larock in Comprehensive Organic Transformations, VCH publishers, 1989.

It may be necessary to protect reactive functional groups (e.g. hydroxy, amino, thio or carboxy) in intermediates used in the preparation of compounds of formula (I) to avoid their unwanted participation in a reaction leading to the formation of compounds of formula (I). Conventional protecting groups, for example those described by T. W. Greene and P. G. M. Wuts in "Protective Groups in Organic Chemistry" John Wiley and Sons. 1999, may be used.

Preparation of compounds of formula (I)

Compounds of formula (I) may be prepared from the corresponding carboxylic acids of formula (II) as shown in Reaction Scheme 1:

Reaction Scheme 1



(II) (III) (I)

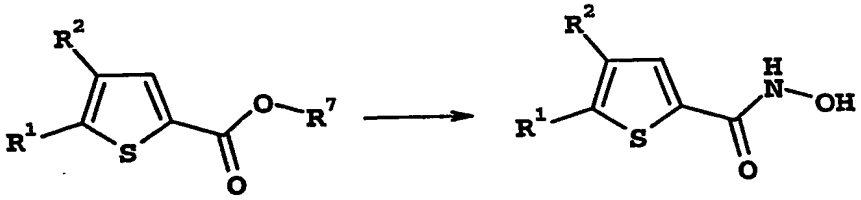
Thus for example a compound of formula (II), wherein R^1 and R^2 are as hereinbefore defined, is reacted, in step 1, with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine and a suitable coupling agent, such as *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate, in the presence of diisopropylethylamine, in an inert solvent, such as dimethylformamide, and at a temperature of about room temperature. The resulting product of formula (III), wherein R^1 and R^2 are as hereinbefore defined, is reacted, in step 2, with an acid catalyst, such as *p*-toluene sulfonic acid, in methanol and at a temperature of about room temperature to obtain compounds of formula (I), wherein R^1 and R^2 are as hereinbefore defined.

Alternatively compounds of formula (I) may be prepared from compounds of formula (II) by reaction with other *O*-protected hydroxylamines, such as *O*-(trimethylsilyl)hydroxylamine, *O*-(*t*-butyldimethylsilyl)-hydroxylamine, or *O*-benzylhydroxylamine, followed by a deprotection using a suitable reagent such as tetra-n-butylammonium fluoride or hydrogen in the presence of a palladium (0) catalyst.

Alternatively compounds of formula (I) may be prepared from compounds of formula (II) by reaction with hydroxylamine.

Compounds of formula (I) may also be prepared from the corresponding esters (IV) as shown in Reaction Scheme 2:

Reaction Scheme 2



(IV) (I)

Thus compounds of formula (IV), wherein R^1 and R^2 are hereinbefore defined and R^7 is lower alkyl (preferably methyl or ethyl), may be reacted with hydroxylamine

hydrochloride in the presence of a base, for example triethylamine, in a protic solvent such as methanol or ethanol at temperatures from room temperature up to the reflux temperature of the solvent to obtain compounds of formula (I), wherein R^1 and R^2 are as hereinbefore defined.

Compounds of formula (I) may also be prepared by interconversion of other compounds of the invention.

As one example, compounds of formula (I) in which R^1 is heteroaryl containing an imino group substituted by alkyl, arylalkyl, or heteroarylalkyl (e.g. R^1 is 1-benzyl-1*H*-pyrazol-3-yl) may be prepared by alkylation of the corresponding compounds of formula (I) in which R^1 is heteroaryl containing an unsubstituted imino group (e.g. R^1 is 1*H*-pyrazol-3-yl) with the appropriate alkyl, arylalkyl- or heteroarylalkyl-halides, preferably bromides, using standard alkylation conditions. The alkylation may for example be carried out in the presence of a base, such as an alkali metal carbonate, e.g. potassium carbonate, or alkali metal hydride, e.g. sodium hydride, in an inert solvent, such as tetrahydrofuran, dimethylformamide or dimethyl sulfoxide, at a temperature from about 0°C to about 100°C.

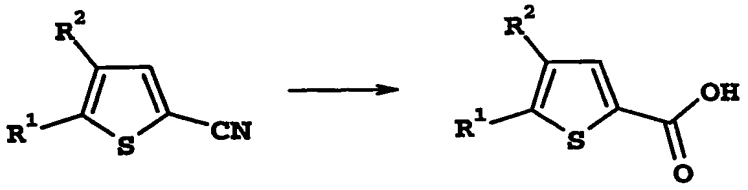
As another example, compounds of formula (I) in which R^1 is heteroaryl containing an *N*-oxide group (e.g. pyridine-*N*-oxide) may be prepared by oxidation of compounds of formula (I) in which R^1 is the corresponding non-oxidised heteroaryl. The oxidation may conveniently be carried out by means of reaction with a mixture of hydrogen peroxide and an organic acid, e.g. acetic acid, preferably at or above room temperature, for example at a temperature of about 60-90°C. Alternatively, the oxidation may be carried out by reaction with a peracid, for example peracetic acid or *m*-chloroperoxybenzoic acid, in an inert solvent such as chloroform or dichloromethane, at a temperature from about room temperature to reflux, preferably at elevated temperature. The oxidation may alternatively be carried out by reaction with hydrogen peroxide in the presence of sodium tungstate at temperatures between room temperature and about 60°C.

The starting materials and intermediates may be prepared by the application or adaptation of methods described herein, or those known in the literature.

Preparation of intermediates of formula (II)

Intermediates of formula (II) may be prepared from compounds of formula (1) as shown in Reaction Scheme 3:

Reaction Scheme 3

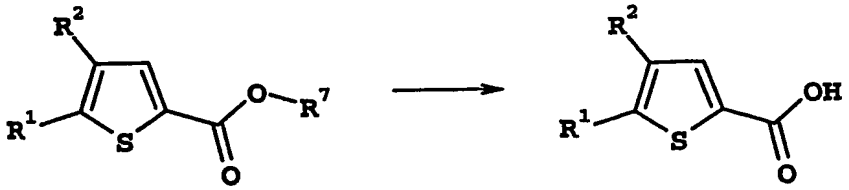


(1) (II)

Thus compounds of formula (1), wherein R^1 and R^2 are as hereinbefore defined, may be reacted with aqueous base, for example sodium hydroxide solution, in a protic solvent, for example methanol or ethanol, at reflux temperature to obtain acids of formula (II), wherein R^1 and R^2 are as hereinbefore defined.

Intermediates of formula (II) may also be prepared from compounds of formula (IV) as shown in Reaction Scheme 4:

Reaction Scheme 4

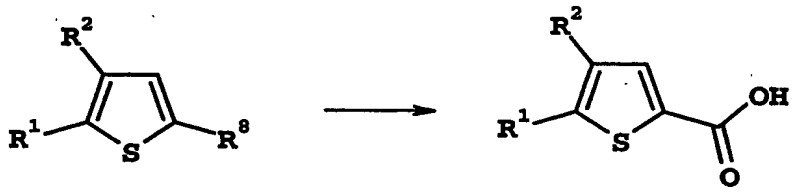


(IV) (II)

Thus compounds of formula (IV), where R^1, R^2 and R^7 are hereinbefore defined, may be reacted with aqueous base, for example sodium hydroxide solution, in a protic solvent, for example methanol or ethanol, at temperatures from room temperature up to reflux temperature to obtain compounds of formula (II), where R^1 and R^2 are hereinbefore defined.

Intermediates of formula (II) may also be prepared from compounds of formula (2) as shown in Reaction Scheme 5:

Reaction Scheme 5



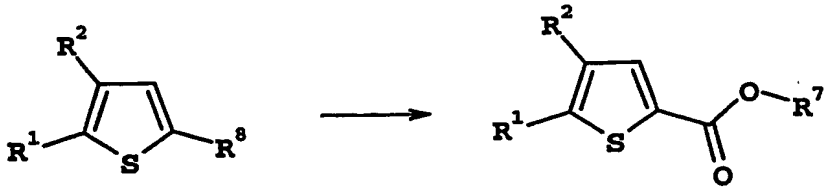
(2) (II)

Thus compounds of formula (2), where R^1 and R^2 are hereinbefore defined and R^8 is hydrogen, bromo, or iodo, may be reacted with an organolithium (for example butyllithium) in an inert solvent (for example diethyl ether or tetrahydrofuran) at temperatures from about room temperature to about - 80°C, followed by reaction with carbon dioxide to obtain compounds of formula (II), where R^1 and R^2 are hereinbefore defined.

Preparation of intermediates of formula (IV)

Intermediates of formula (IV) may be prepared from compounds of formula (2) as shown in Reaction Scheme 6:

Reaction Scheme 6



(2) (IV)

Thus compounds of formula (2), where R^1 and R^2 are hereinbefore defined and R^8 is hydrogen, bromo, or iodo, may be reacted with an organolithium (for example butyllithium) in an inert solvent (for example diethyl ether or tetrahydrofuran) at temperatures from about room temperature to about - 80°C, followed by reaction with an alkyl chloroformate of formula R^7-O-C(=O)-Cl, wherein R^7 is as hereinbefore defined, (e.g. methyl chloroformate or ethyl chloroformate) to obtain compounds of formula (IV), where R^1, R^2 and R^7 are hereinbefore defined.

Preparation of intermediates of formula (1)

Compounds of formula (1) may be prepared from compounds of formula (3) as shown in Reaction Scheme 7:

Reaction Scheme 7

(3) (1)

Thus compounds of formula (3), wherein R^1 and R^2 are as hereinbefore defined and R^9 is bromo or iodo, may be reacted with copper (1) cyanide in an inert solvent such as *N,N*-dimethylformamide, or *N*-methyl-2-pyrrolidinone, at elevated temperatures from

about 100°C up to the reflux temperature of the solvent to obtain compounds of formula (1), wherein R^1 and R^2 are as hereinbefore defined.

Alternatively, compounds of formula (1) may be prepared from compounds of formula (3) by reaction with zinc (2) cyanide in the presence of a palladium (0) catalyst, for example tetrakis (triphenylphospine)palladium (0), in an inert solvent, for example *N,N*-dimethylformamide, at temperatures from about room temperature up to reflux temperature.

Preparation of intermediates of formula (3)

Intermediates of formula (3) may be prepared from compounds of formula (4) as shown in Reaction Scheme 8:

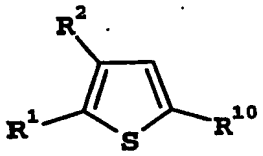
Reaction Scheme 8

(4) (3)

Thus compounds of formula (3), wherein R^1 and R^2 are as hereinbefore defined and R^9 is bromo or iodo, may be prepared from compounds of formula (4), wherein R^1 and R^2 are as hereinbefore defined, by reaction with an appropriate halogenating agent, for example bromine, iodine, *N*-bromosuccinimide, or *N*-iodosuccinimide.

General Methods for the Preparation of Compounds of formulae (II), (IV), (1), and (4)

Common synthetic methods may be applied to compounds of formula (5), where R^{10} is hydrogen, carboxy, $C(=O)OR^7$ or cyano:

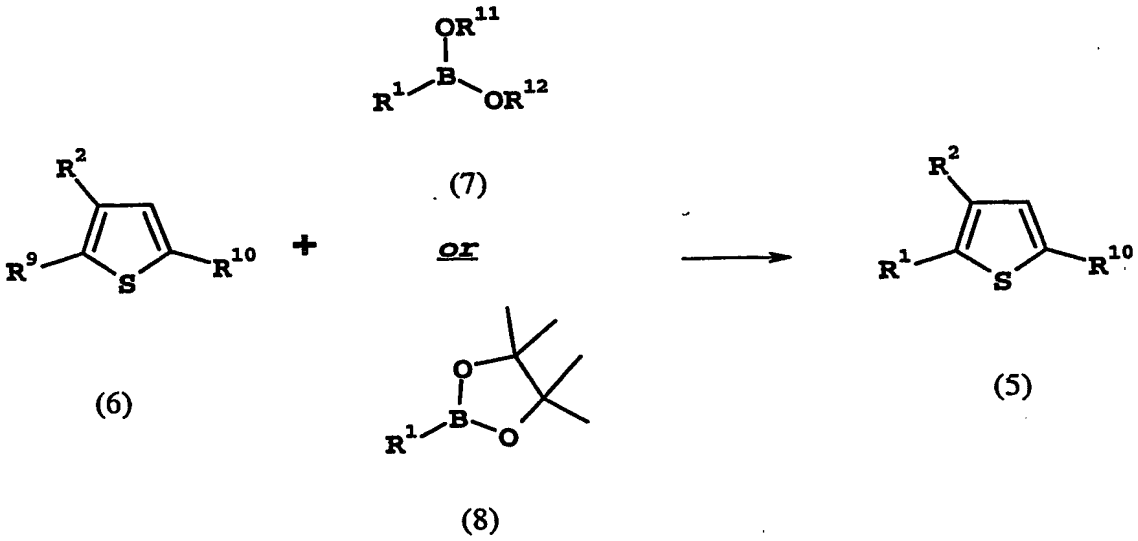


(5)

It should be understood that formula (5) is a general formula which comprises compounds of formulae (II), (IV), (1), and (4).

Compounds of formula (5) may be prepared from compounds of formula (6) as shown in Reaction Scheme 9:

Reaction Scheme 9



(6) (7) or (8) (5)

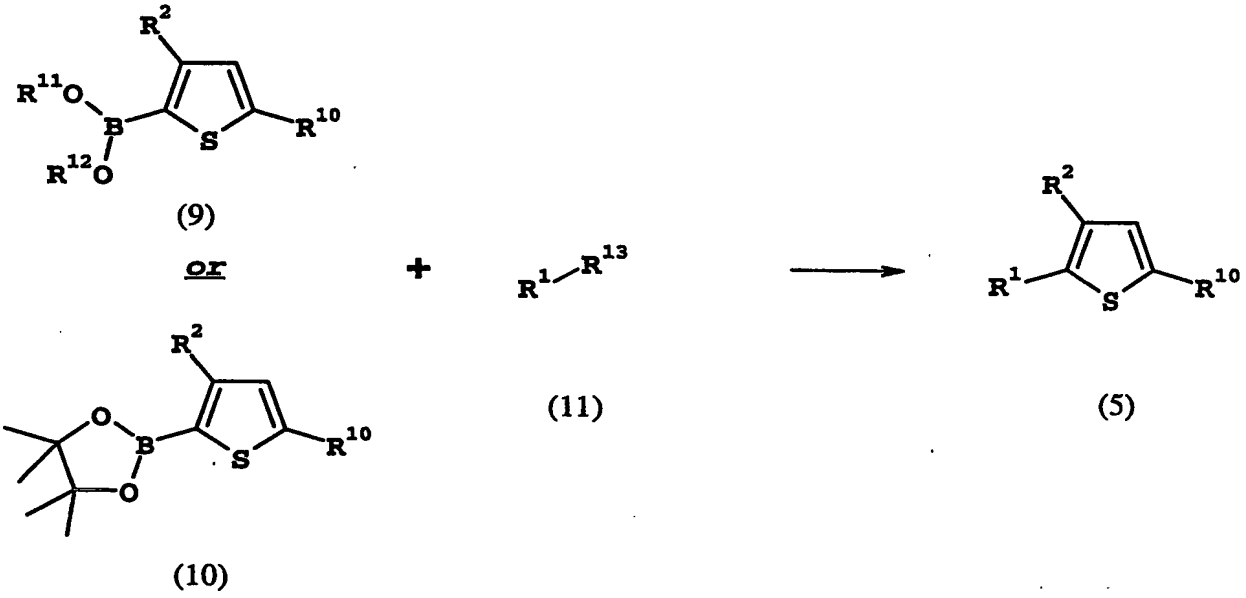
Thus compounds of formula (6), wherein R^2 and R^{10} are as hereinbefore defined and R^9 is bromo or iodo, may be coupled with compounds of formula (7), in which R^1 is hereinbefore defined and R^{11} and R^{12} are independently hydrogen or lower alkyl, to obtain compounds of formula (5), wherein R^1, R^2 and R^{10} are as hereinbefore defined. The reaction is performed in the presence of a suitable catalyst, such as tetrakis(triphenylphosphine)palladium (0), and a suitable base, such as cesium carbonate in

a suitable solvent such as *N,N*-dimethylformamide at a temperature of from about room temperature up to the reflux temperature of the solvent.

Alternatively the coupling reaction may be carried out using compounds of formula (8), wherein R^1 is as hereinbefore defined.

Compounds of formula (5) may also be prepared from compounds of formula (11) as shown in Reaction Scheme 10:

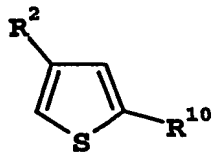
Reaction Scheme 10:



Thus compounds of formula (11), wherein R^1 is as hereinbefore defined and R^{13} is bromo, iodo, or trifluoromethanesulfonyloxy, may be reacted with compounds of formula (9), wherein R^2 and R^{10} are as hereinbefore defined and R^{11} and R^{12} are independently hydrogen or lower alkyl, to obtain compounds of formula (5), wherein R^1, R^2 and R^{10} are as hereinbefore defined. The reaction is performed in the presence of a suitable catalyst, such as tetrakis(triphenylphosphine)palladium (0), and a suitable base, such as cesium carbonate, in a suitable solvent, such as *N,N*-dimethylformamide, and at a temperature from about room temperature up to the reflux temperature of the solvent.

Alternatively, the coupling reaction may also be carried out using compounds of formula (10) wherein R^2 and R^{10} are as hereinbefore defined.

Compounds of formula (6), wherein R^2 and R^{10} are as hereinbefore defined and R^9 is bromo or iodo, may be prepared from compounds of formula (12):-



(12)

wherein R^2 and R^{10} are as hereinbefore defined, by reaction with a suitable halogenating agent such as bromine, iodine, *N*-bromosuccinimide, or *N*-iodosuccinimide.

Compounds of formula (7), wherein R^1, R^{11} and R^{12} are as hereinbefore defined, may be obtained from commercial sources. Alternatively, compounds of formula (7), wherein R^1 is as hereinbefore defined and R^{11} and R^{12} are both methyl (or ethyl), may be may be obtained by, for example, the reaction of an organometallic reagent of formula (13):-

$$R^1—M$$

(13)

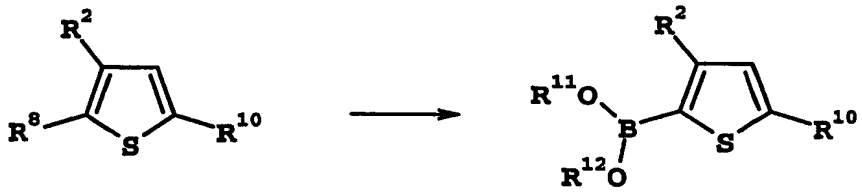
where R^1 is as previously defined and M is a metal atom such as lithium or magnesium, with trimethylborate (or triethylborate).

Compounds of formula (8), wherein R^1 is as hereinbefore defined, may be prepared from compounds of formula (11), wherein R^1 and R^{13} are as hereinbefore defined, by reaction with bis(pinacolato)diboron in the presence of a suitable catalyst, such as tetrakis(tri-phenylphosphine)palladium (0), and a suitable base, such as cesium carbonate in a suitable

solvent such as *N*,*N*-dimethylformamide at a temperature of from about room temperature up to the reflux temperature of the solvent.

Compounds of formula (9) may be prepared according to Reaction Scheme 11:

Reaction Scheme 11:



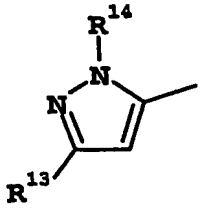
(14) (9)

Thus compounds of formula (14), wherein R^2 and R^{10} are as hereinbefore defined and R^8 is hydrogen, bromo, or iodo, may be reacted with an organolithium reagent, for example butyllithium, followed by reaction with trimethylborate (or triethylborate), in an inert solvent such as tetrahydrofuran, at temperatures from about – 80°C to about room temperature to obtain compounds of formula (9), wherein R^2 and R^{10} are as hereinbefore defined and R^{11} and R^{12} are both methyl (or ethyl).

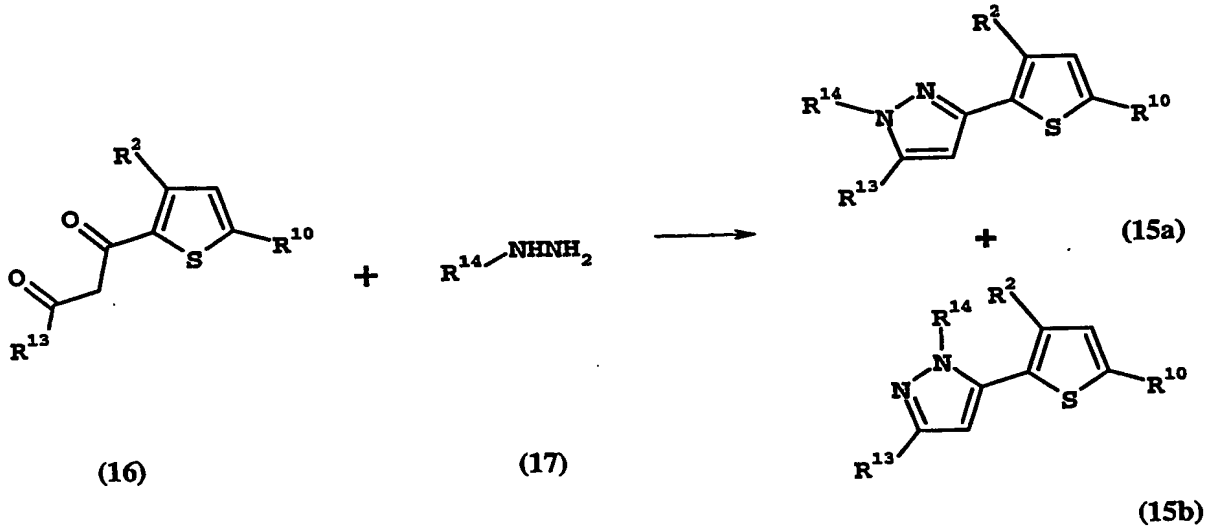
Compounds of formula (10), wherein R^2 and R^{10} are as hereinbefore defined, may be prepared from compounds of formula (6), wherein R^2, R^9 and R^{10} are as hereinbefore defined by reaction with bis(pinacolato)diboron in the presence of a suitable catalyst, such as tetrakis(triphenylphosphine)palladium (0), and a suitable base, such as cesium carbonate in a suitable solvent such as *N*,*N*-dimethylformamide at a temperature of from about room temperature up to the reflux temperature of the solvent.

Compounds of formula (11) may be obtained from commercial sources, or may be prepared using published methods described in the literature.

Compounds of formula (12) may be obtained from commercial sources, or may be prepared using published methods described in the literature.

Compounds of formula (5), wherein R^2 is as hereinbefore defined, R^{10} is hydrogen or cyano and R^1 is  or  (in which R^{13} is hydrogen, trifluoromethyl, alkyl, aryl, heteroaryl, heterocycloalkyl, arylalkyl, heteroarylalkyl, or heterocycloalkylalkyl and R^{14} is hydrogen, alkyl, aryl, heteroaryl, heterocycloalkyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroarylalkyl or heterocycloalkylalkyl), hereinafter described as compounds of formula (15a) and (15b), may be prepared according to Reaction Scheme 12:

Reaction Scheme 12

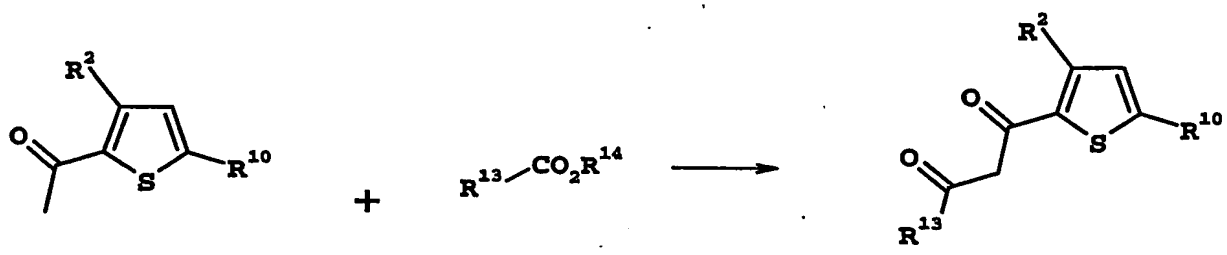


(16) (17) (15a) (15b)

Thus 1,3-diketones of formula (16), wherein R^2 and R^{13} are as hereinbefore defined, and R^{10} is hydrogen or cyano, may be reacted with hydrazines of formula (17), wherein R^{14} is as hereinbefore defined, to obtain compounds of formula (15a) and (15b). The reaction may be carried out in a protic solvent, for example an alcohol, preferably ethanol, at temperatures from about room temperature up to the reflux temperature of the solvent. It will be recognized that such reactions may give rise to mixtures of the two regioisomers (15a) and (15b), the ratio of which will depend upon the nature of the groups R^2, R^{13}, and R^{14}, and the reaction conditions. Where produced, such regioisomers may be separated by classical techniques such as fractional crystallisation or chromatography.

Compounds of formula (16), wherein R^2 and R^{13} are as hereinbefore defined and R^{10} is hydrogen or cyano, may be prepared as shown in Reaction Scheme 13:

Reaction Scheme 13

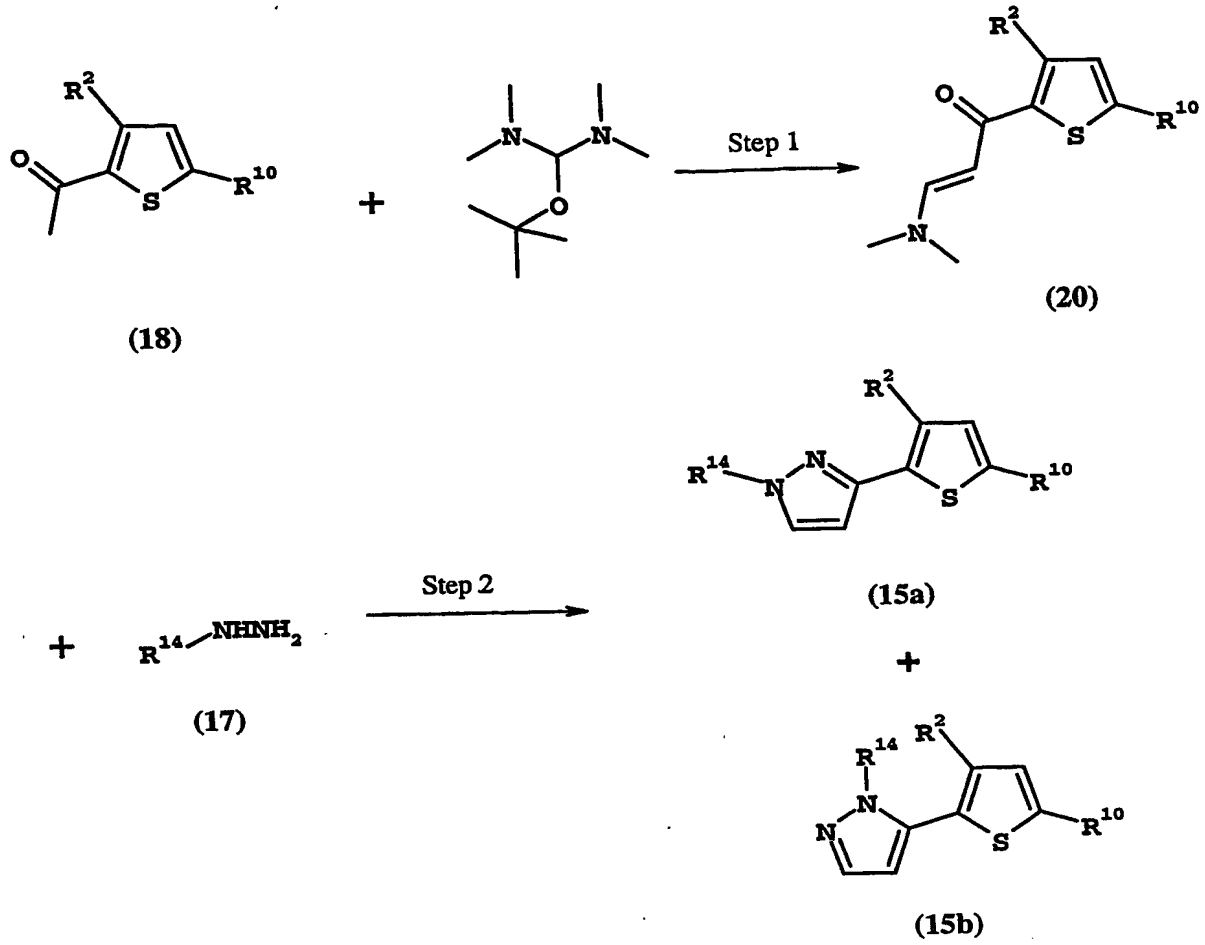


(18) (19) (16)

Thus compounds of formula (18), wherein R^2 is as hereinbefore defined and R^{10} is hydrogen or cyano, may be reacted with compounds of formula (19), wherein R^{13} is as hereinbefore defined and R^{14} is lower alkyl, to obtain compounds of formula (16). The reaction may conveniently be carried out with a suitable base, for example sodium methoxide, in a protic solvent such as an alcohol, for example methanol, at temperatures of from about room temperature up to the reaction temperature of the solvent.

Compounds of formula (15a) and (15b), where R^{10} is cyano and R^{13} is H, may be prepared as shown in Reaction Scheme 14:

Reaction Scheme 14



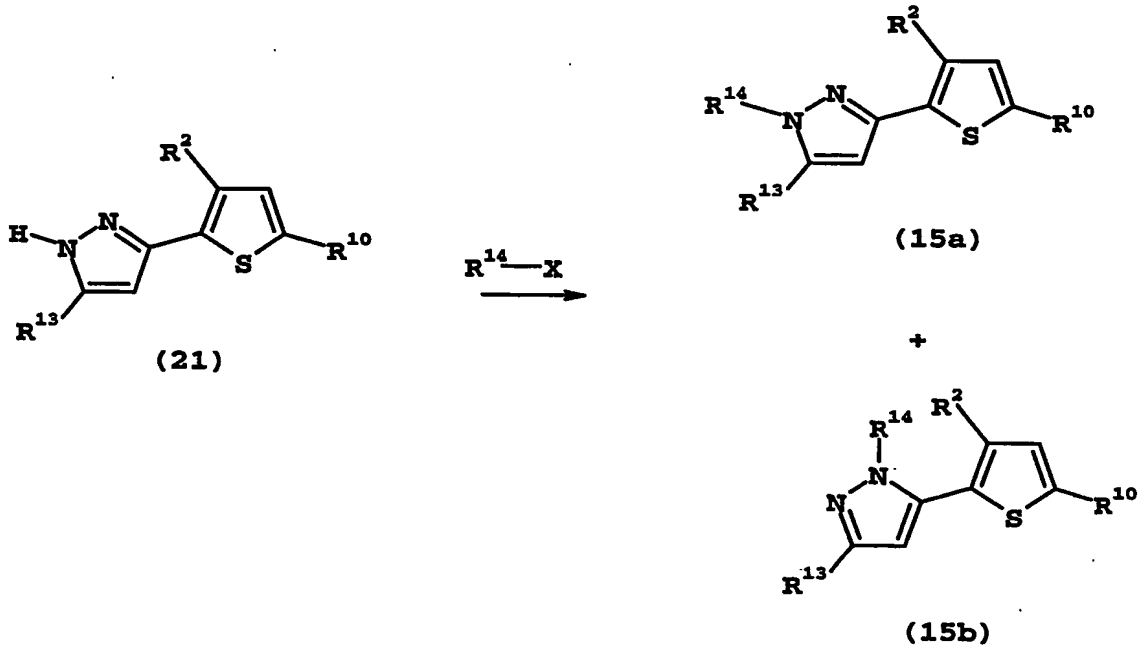
Thus for example compounds of formula (18), wherein R^2 is as hereinbefore defined and R^{10} is cyano, may be reacted, in step 1, with *tert*-butoxybis(dimethylamino)methane in a suitable solvent such as *N*,*N*-dimethylformamide at temperatures of from about room temperature up to about the reflux temperature of the solvent. The resulting intermediate of formula (20), wherein R^2 is as hereinbefore defined and R^{10} is cyano, may be reacted, in step 2, with hydrazines of formula (17), wherein R^{14} is as described hereinbefore, to obtain compounds of formula (15a) and (15b), wherein R^2 and R^{14} are as hereinbefore described and R^{10} is cyano. Step 2 may conveniently be carried out in a protic solvent, for example an alcohol, preferably ethanol, at temperatures from about room temperature up to the reflux temperature of the solvent. It will be recognized that such reactions may give rise to two regioisomers, the ratio of which will depend upon the nature of the groups R^2

and R^{14}, and the reaction conditions. Where produced, such regioisomers may be separated by classical techniques such as fractional crystallisation or chromatography.

Compounds of formula (17) and (18) are commercial or are described in the literature.

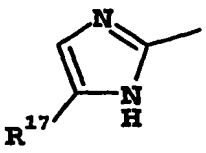
Compounds of formula (15a) and (15b), where R^{14} is alkyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocycloalkylalkyl, may be prepared as shown in Reaction Scheme 15:

Reaction Scheme 15

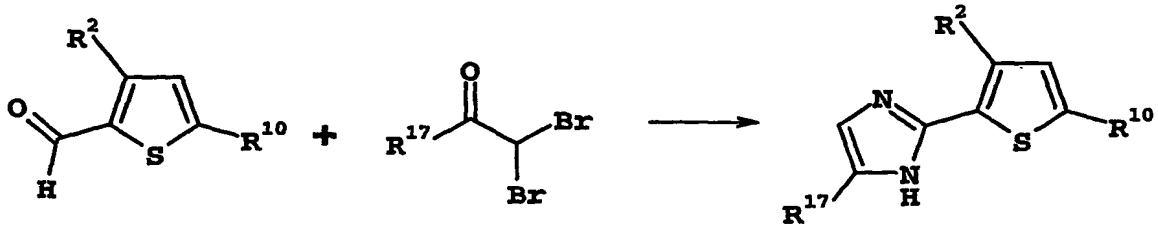


Thus for example compounds of formula (21), wherein R^2, R^{10}, and R^{13} are as hereinbefore defined, may be reacted with compounds of formula R^{14}-X, wherein R^{14} is alkyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocycloalkylalkyl and X is halo (preferably bromo) or $-OSO_2CH_3$, in the presence of a suitable base, for example sodium hydride, in an inert solvent such as *N,N*-dimethylformamide at temperatures of from about room temperature up to the reflux temperature of the solvent. It will be recognized that such reactions may give rise to two regioisomers, the ratio of which will depend upon the nature of the groups R^2, R^{13}, and R^{14}, and the reaction conditions. Where produced, such

regioisomers may be separated by classical techniques such as fractional crystallisation or chromatography.

Compounds of general formula (5), where R^{10} is hydrogen, carboxy, $C(=O)OR^7$ or cyano, and R^1 is  in which R^{17} is trifluoromethyl, alkyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl or heterocycloalkylalkyl, hereinafter described as compounds of formula (22), may be prepared as shown in Reaction Scheme 16:

Reaction scheme 16



(23) (24) (22)

Thus compounds of formula (23) may be reacted with compounds of formula (24), wherein R^{17} is as hereinbefore defined, to obtain the said compounds of formula (22). The reaction may conveniently be carried in an aqueous alcoholic solvent, for example aqueous methanol, in the presence of a suitable buffer, for example ammonium acetate, at temperatures of from about room temperature to about the reflux temperature of the solvent.

The compositions of the present invention may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers or excipients. Thus, the active compounds of the invention may be formulated for oral, buccal, intranasal, parenteral (*e.g.*, intravenous, intramuscular or subcutaneous) transdermal or rectal administration or in a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.* pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropylmethylcellulose); fillers (*e.g.* lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.* magnesium stearate, talc or silica); disintegrants (*e.g.* potato starch or sodium starch glycollate); or wetting agents (*e.g.* sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.* sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (*e.g.* lecithin or acacia); non-aqueous vehicles (*e.g.* almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.* methyl or propyl p-hydroxybenzoates or sorbic acid).

For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

The active compounds of the invention may be formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form e.g. in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilising and/or dispersing agents.

Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, *e.g.* sterile pyrogen-free water, before use.

The active compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds of the invention are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, *e.g.* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

A proposed dose of the active compounds of the invention for oral, parenteral or buccal administration to the average adult human for the treatment of the conditions referred to above is 0.1 to 500 mg of the active ingredient per unit dose which could be administered, for example, 1 to 4 times per day.

The invention will now be described in detail with reference to the following examples. It will be appreciated that the invention is described by way of example only and modification of detail may be made without departing from the scope of the invention.

EXPERIMENTAL

400MHz ^{1}H nuclear magnetic resonance spectra (NMR) were recorded at ambient temperature using a Varian Unity Inova (400MHz) spectrometer with a triple resonance 5mm probe. In the NMR chemical shifts (δ) are expressed ppm relative to tetramethylsilane. The following abbreviations have been used: br = broad signal, s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, dt = double triplet, t = triplet, td = triple doublet, q = quartet.

High Pressure Liquid Chromatography - Mass Spectrometry (LCMS) experiments to determine retention times (R_T) and associated mass ions were performed using one of the following methods.

Method A: Experiments performed on a Micromass Platform LCT spectrometer with positive ion electrospray and ELS/Single wavelength UV 254nm detection using a Higgins Clipius C18 5µm 100x3.0mm column and a 2 ml / minute flow rate. The initial solvent system was 95% water containing 0.1% formic acid (solvent A) and 5% acetonitrile containing 0.1% formic acid (solvent B) for the first minute followed by a gradient up to 5% solvent A and 95% solvent B over the next 14 minutes. The final solvent system was held constant for a further 2 minutes.

Method B: Experiments performed on a Micromass Platform LC spectrometer with positive and negative ion electrospray and ELS/Diode array detection using a Waters XTerra MS C18 3.5µm 30x4.6mm column and a 2 ml / minute flow rate. The solvent system was 95% solvent A and 5% solvent B for the first 0.25 minutes followed by a gradient up to 5% solvent A and 95% solvent B over the next 2 minutes. The final solvent system was held constant for a further 0.25 minutes.

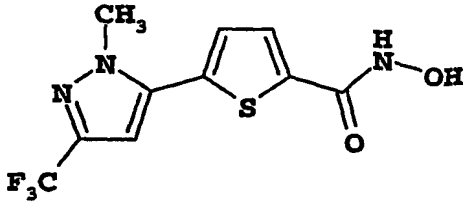
Method C: Experiments performed on a Micromass Platform LC spectrometer with positive and negative ion electrospray and ELS/Diode array detection using a Phenomenex Luna C18(2) 30 x 4.6mm column. The solvent system was 95% solvent A and 5% solvent B for the first 0.50 minutes followed by a gradient up to 5% solvent A and 95% solvent B over the next 4 minutes. The final solvent system was held constant for a further 0.50 minutes.

Reverse Phase High Pressure Liquid Chromatography purification was performed using a Genesis HPLC Column (Ref. 16R10985, 100mmx22.5mm) containing C18-7µm 120A silica.

TLC analysis was performed on Fluka aluminium-backed silica gel/TLC cards (20x20cm) with layer thickness 0.2mm, cut to size.

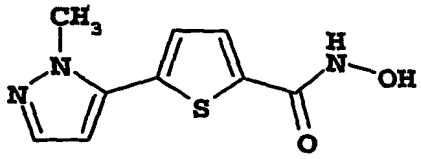
EXAMPLE 1

(a) 5-(2-Methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



A solution of 5-(2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [29mg, 0.08mmol, Reference Example 1(a)] in methanol (0.8ml) was treated with *p*-toluene sulfonic acid (0.7mg, 0.003mmol). The solution was stirred at room temperature for 1 hour when t.l.c. [ethyl acetate/petroleum ether (b.p. 40-60°C), 3:2, v/v] indicated complete disappearance of the starting material. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The two phases were separated and the organic phase was washed with water, then dried over sodium sulphate and then evaporated under reduced pressure to give 5-(2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (22mg, 96%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.53 (br, 1H), 7.23 (br, 1H), 6.79 (br, 1H), 4.00 (s, 3H).. LCMS (Method A): R_T = 6.45 minutes; 292 $(M+H)^+$.

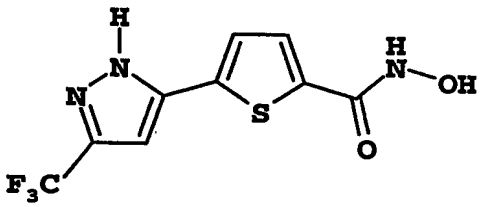
(b) 5-(2-Methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(b)] there was prepared a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (45mg, 91%). This was subjected to reverse-phase preparative HPLC (gradient elution, 5% acetonitrile/water to 95% acetonitrile/water over 90 minutes) to provide 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (16mg, 32%) as the more mobile fraction as an off-white solid. ^{1}H NMR

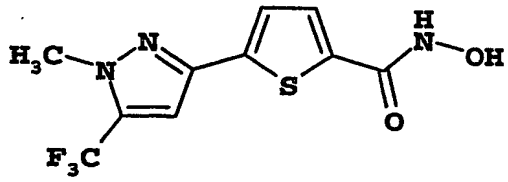
(CD_3OD): δ 7.61 (br, 1H), 7.49 (d, *J*=2Hz, 1H), 7.32 (d, *J*=4Hz, 1H), 6.53 (d, *J*=2Hz, 1H), 3.99 (s, 3H). LCMS (Method A): R_T = 3.96 minutes; 224 $(M+H)^+$.

(c) 5-(5-Trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



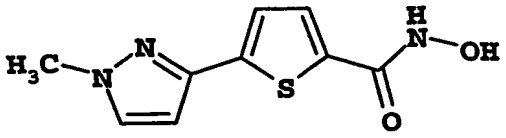
By proceeding in a similar manner to Example 1(a) but using 5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(c)] there was prepared 5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (3mg, 11%) as a fawn coloured solid. ^1H NMR [$(CD_3)_2SO$]: δ 11.36 (br, 1H), 9.24 (s, 1H), 7.62 (br, 1H), 7.54 (d, *J*=4.0Hz, 1H), 7.15 (s, 1H). LCMS (Method A): R_T = 5.81 minutes; 278 $(M+H)^+$.

(d) 5-(1-Methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



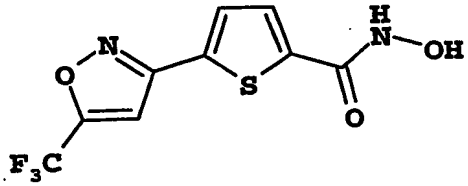
By proceeding in a similar manner to Example 1(a) but using 5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(d)] there was prepared 5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide as a white solid (80mg, 95%). ^1H NMR [$(CD_3)_2SO$]: δ 11.41 (s br, 1H), 9.27 (br, 1H), 7.68 (d br, *J*=3.9Hz, 1H), 7.53 (d, *J*=3.9Hz, 1H), 7.11 (s, 1H), 4.05 (s, 3H). LCMS (Method A): R_T = 6.41 minutes; 292 $(M+H)^+$.

(e) 5-(1-Methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



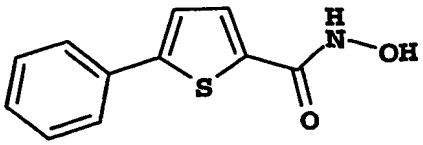
By proceeding in a similar manner to Example 1(a) but using a mixture of 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide and 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(b)] there was prepared 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (15mg, 72%) as pale brown oil. ^{1}H NMR (CD_3OD): δ 7.61 (d, *J*=2.3Hz, 1H), 7.52 (br, 1H), 7.32 (d, *J*=3.9Hz, 1H), 6.58 (d, *J*=2.3Hz, 1H), 3.91 (s, 3H).

(f) 5-(5-Trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(e)] there was prepared 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (16mg, 95%) as an off-white solid. ^{1}H NMR [$(CD_3)_2CO$]: δ 10.85 (s br, 1H), 8.49 (br, 1H), 7.80 (d, *J*=3.7Hz, 1H), 7.76 (br, 1H), 7.75 (s, 1H). LCMS (Method A): R_T = 6.84 minutes; 279 $(M+H)^+$.

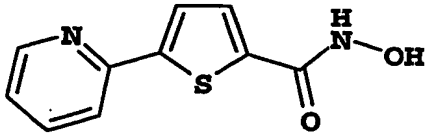
(g) 5-Phenyl-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-phenyl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [80mg, Reference Example 1(f)] and washing the white solid obtained after evaporation of the reaction mixture, with water, then twice with dichloromethane, then with saturated sodium bicarbonate solution, then twice with ether and then drying under vacuum there was prepared 5-phenyl-thiophene-2-

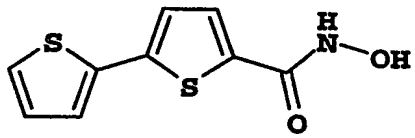
carboxylic acid hydroxyamide (31mg, 54%) as a white solid. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.20 (s br, 1H), 9.10 (s br, 1H), 7.65 (d, *J*=8Hz, 2H), 7.55 (br, 1H), 7.47 (d, *J*=4.0Hz, 1H), 7.40 (t, *J*=8Hz, 2H), 7.31 (t, *J*=8Hz, 1H). LCMS (Method A): R_T = 6.29 minutes; 220 $(M+H)^+$.

(h) 5-Pyridin-2-yl-thiophene-2-carboxylic acid hydroxyamide



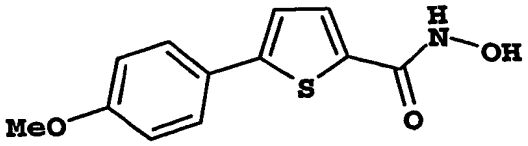
By proceeding in a similar manner to Example 1(a) but using 5-pyridine-2-yl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [228mg, 0.75mmol, Reference Example 1(g)] there was prepared 5-pyridin-2-yl-thiophene-2-carboxylic acid hydroxyamide (14mg, 8%) as a yellow solid. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.27 (s, 1H), 9.16 (s, 1H), 8.57 (ddd, *J*=4.9, 1.7, 0.9Hz, 1H), 7.96 (dt, *J*=7.9, 0.9, 0.9Hz, 1H), 7.87 (td, *J*=7.9, 7.5, 1.7Hz, 1H), 7.79 (d, *J*=4.0Hz, 1H), 7.62 (br, 1H), 7.34 (ddd, *J*=7.5, 4.9, 0.9Hz, 1H). LCMS (Method A): R_T = 4.11 minutes; 221 $(M+H)^+$.

(i) [2,2']Bithiophenyl-5-carboxylic acid hydroxyamide



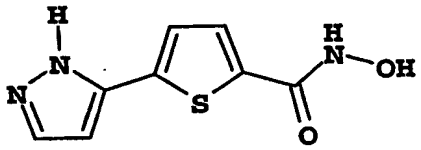
By proceeding in a similar manner to Example 1(a) but using [2,2']bithiophenyl-5-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(h)] and subjecting the reaction mixture to column chromatography there was prepared [2,2']bithiophenyl-5-carboxylic acid hydroxyamide (54mg, 38%) as a brown solid. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.27 (s br, 1H), 9.17 (s br, 1H), 7.59 (d, *J*=5.1Hz, 1H), 7.55 (br, 1H), 7.41 (d, *J*=3.4Hz, 1H), 7.30 (d, *J*=3.7Hz, 1H), 7.12 (dd, *J*=5.1, 3.7Hz, 1H). LCMS (Method A): R_T = 5.99 minutes; 226 $(M+H)^+$.

(j) 5-(4-Methoxy-phenyl)-thiophene-2-carboxylic acid hydroxyamide



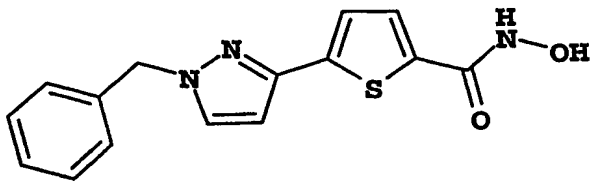
By proceeding in a similar manner to Example 1(a) but using 5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(i)] there was prepared 5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid hydroxyamide (78mg, 96%) as a pale yellow solid. ^{1}H NMR (CD_3OD): δ 7.60 (d, *J*=8.8Hz, 2H), 7.53 (br, 1H), 7.26 (d, *J*=4.0Hz, 1H), 6.97 (d, *J*=8.8Hz, 2H), 3.82 (s, 3H). LCMS (Method A): R_T = 6.39 minutes; 250 $(M+H)^+$.

(k) 5-(2*H*-Pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [120mg, 0.40mmol, Reference Example 1(j)] and subjecting the reaction mixture to reverse-phase HPLC (gradient elution, 5% acetonitrile/water to 95% acetonitrile/water over 90 minutes) there was prepared 5-(2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (79mg, 92%) as a white solid. ^{1}H NMR (CD_3OD): δ 7.69 (d, *J*=2.3Hz, 1H), 7.54 (br, 1H), 7.36 (d, *J*=4.0Hz, 1H), 6.64 (d, *J*=2.3Hz, 1H). LCMS (Method A): R_T = 3.49 minutes; 210 $(M+H)^+$.

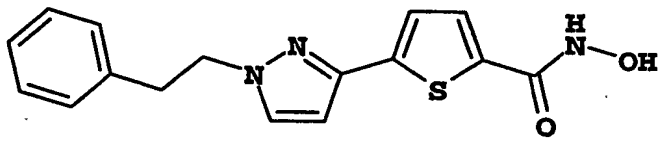
(l) 5-(1-Benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example

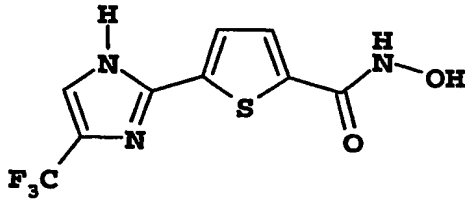
1(k)] and purification of the reaction mixture by preparative reverse-phase HPLC (gradient elution, 5% acetonitrile/water to 95% acetonitrile/water over 90 minutes) there was prepared 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (54mg, 96%) as a pale brown solid. ^{1}H NMR (CD_3OD): δ 7.66 (d, *J*=2.3Hz, 1H), 7.52 (br, 1H), 7.24-7.36 (m, 6H), 6.62 (d, *J*=2.3Hz, 1H), 5.35 (s, 2H). LCMS (Method A): R_T = 6.54 minutes; 300 $(M+H)^+$.

(m) 5-(1-Phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [Reference Example 1(l)] there was prepared 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide (121mg, 97%) as a pale brown solid. ^{1}H NMR (CD_3OD): δ 7.53 (br, 1H), 7.39 (d, *J*=2.3Hz, 1H), 7.32 (d, *J*=4.0Hz, 1H), 7.25 (m, 2H), 7.18 (m, 1H), 7.12 (m, 2H), 6.49 (d, *J*=2.3Hz, 1H), 4.37 (t, *J*=7.2Hz, 2H), 3.16 (t, *J*=7.2Hz, 2H). LCMS (Method A): R_T = 7.02 minutes; 314 $(M+H)^+$.

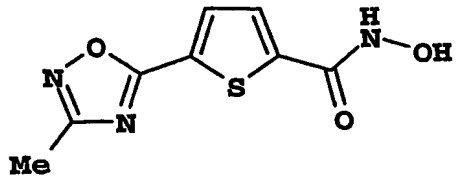
(n) 5-(4-Trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [42mg, 0.15mmol, Reference Example 1(m)] and triturating the reaction mixture with water there was prepared 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid hydroxyamide (8mg, 25%) as a white powder. ^{1}H NMR [$(CD_3)_2SO$]: δ 13.42 (s, 1H),

11.32 (s, 1H), 9.21 (s, 1H), 7.96 (s, 1H), 7.60 (s, 2H). LCMS (Method A): R_T = 4.85 minutes; 278 $(M+H)^+$.

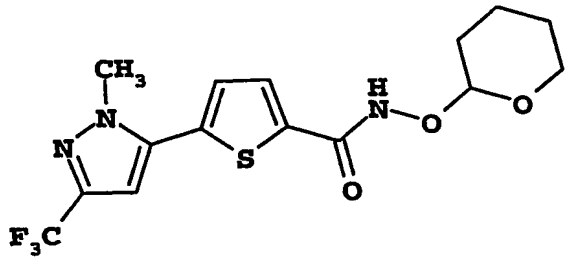
(o) 5-(3-Methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid hydroxyamide



By proceeding in a similar manner to Example 1(a) but using 5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide [155mg, 0.48mmol, Reference Example 1(n)], filtering the resulting precipitate (which was then washed with methanol) there was prepared 5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid hydroxyamide (65mg, 60%) as a white solid. ^{1}H NMR [$(CD_3)_2SO$]: δ 11.60 (s, 1H), 9.41 (s, 1H), 7.98 (d, *J*=4.0Hz, 1H), 7.73 (d br, *J*=4.0Hz, 1H), 2.41 (s, 3H). LCMS (Method A): R_T = 4.26 minutes; 226 $(M+H)^+$.

REFERENCE EXAMPLE 1

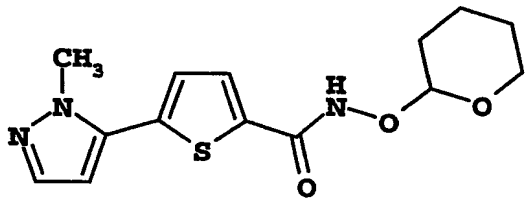
(a) 5-(2-Methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetra-hydro-pyran-2-yloxy)-amide



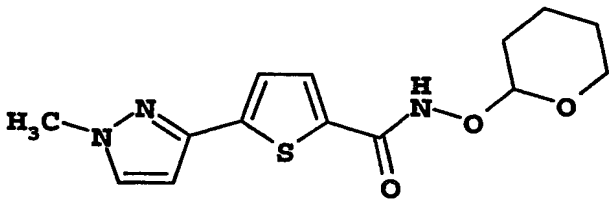
A solution of 5-[2-methyl-5-(trifluoromethyl)-2*H*-pyrazol-3-yl]thiophene-2-carboxylic acid (80mg, 0.29mmol) in dimethylformamide (1.2ml) was treated with diisopropylethylamine (151µl, 0.87mmol), *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (39mg, 0.33mmol) and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (110mg, 0.29mmol). The mixture was stirred at room temperature for 4 hours when t.l.c. analysis [ethyl acetate/methanol, 3:1, v/v] indicated complete consumption of the starting carboxylic acid. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between ethyl acetate and saturated

sodium bicarbonate solution. The two phases were separated and the organic phase was washed with water, then dried over sodium sulphate and then evaporated under reduced pressure. The crude product was subjected to flash column chromatography on silica eluting with a mixture of ethyl acetate and petroleum ether fraction (b.p. 30-50°C), (3:2, v/v), to give 5-(2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (85mg, 78%) as a white solid. LCMS (Method A): R_T = 8.45 minutes; 376 $(M+H)^+$.

(b) 5-(2-Methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide

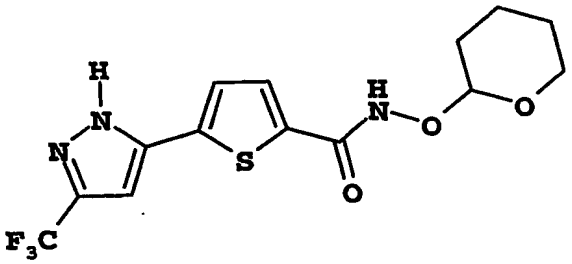


and



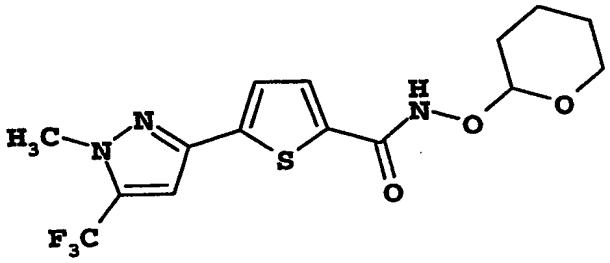
By proceeding in a similar manner to Reference Example 1(a) but using a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid [Reference Example 2(a)] there was prepared a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (75mg, 73%) as a colourless foam. LCMS (Method A): R_T = 5.95 minutes (minor component) and 6.08 minutes (major component); 308 $(M+H)^+$.

(c) 5-(5-Trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



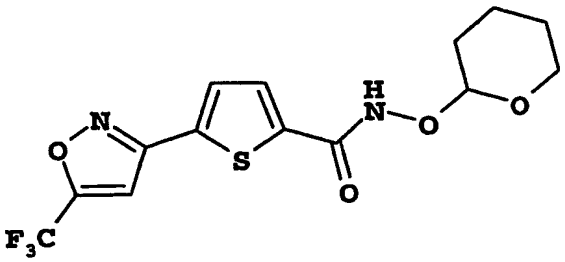
By proceeding in a similar manner to Reference Example 1(a) but using 5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid there was prepared 5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (51mg, 52%) as a white solid. LCMS (Method C): R_T = 3.10 minutes; 362 $(M+H)^+$.

(d) 5-(1-Methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetra-hydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using 5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid there was prepared 5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (244mg, 88%) as a yellow gum. LCMS (Method A): R_T = 8.49 minutes; 376 $(M+H)^+$.

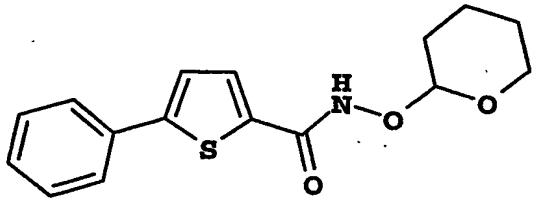
(e) 5-(5-Trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using 5-(5-hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carboxylic acid [Reference Example 2(b)] there was prepared a mixture of 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide and 5-(5-hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide. The mixture was separated by flash chromatography on silica eluting with 28% - 40%(v/v) ethyl acetate in petroleum ether fraction (b.p. 40-60°C) to yield 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (22mg, 23%) as a white solid.

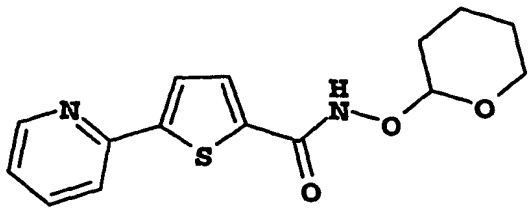
LCMS (Method A): R_T = 8.95 minutes; 363 $(M+H)^+$.

(f) 5-Phenyl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



To a solution of 5-phenyl-thiophene-2-carboxylic acid (72mg, 0.35mmol) in *N*,*N*-dimethylformamide (3ml) at 0°C was added *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (45mg, 0.39mmol), diisopropylethylamine (153µl, 0.88mmol), and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (148mg, 0.39mmol). The mixture was allowed to equilibrate to room temperature over 7 hours. The volatiles were evaporated and the residue was partitioned between ethyl acetate and water. The two phases were separated and the aqueous phase was extracted twice with ethyl acetate. The combined extracts were washed with water, then with 10% citric acid solution, then with saturated sodium bicarbonate solution, then with brine, then dried over magnesium sulphate and then evaporated. The residual yellow gum was subjected to column chromatography on silica eluting with ethyl acetate/ pentane (1:3 v/v) to yield 5-phenyl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (87mg, 81%) as a white gum, which crystallised on standing. LCMS (Method A): R_T = 8.48 minutes; 304 $(M+H)^+$.

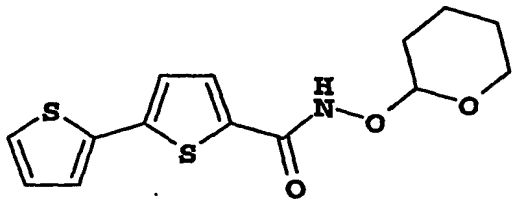
(g) 5-Pyridin-2-yl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(f) but using 5-pyridin-2-yl-thiophene-2-carboxylic acid there was prepared 5-pyridin-2-yl-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (233mg, 78%) as a pale yellow gum.

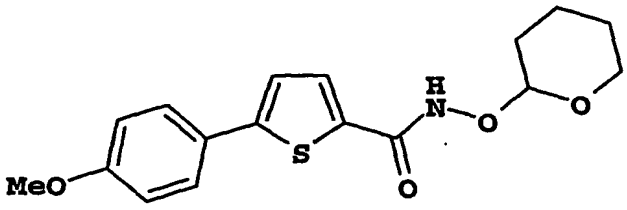
LCMS (Method A): R_T = 6.32 minutes; 305 $(M+H)^+$.

(h) [2,2']Bithiophenyl-5-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using [2,2']bithiophenyl-5-carboxylic acid there was prepared [2,2']bithiophenyl-5-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (212mg, 79%) as a colourless oil, which was used in the next step without further purification.

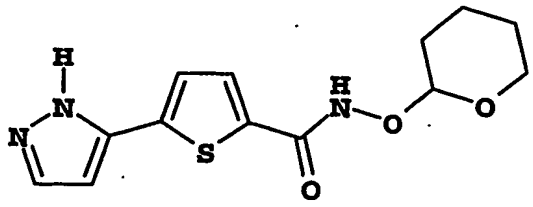
(i) 5-(4-Methoxy-phenyl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using 5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid there was prepared 5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (195mg, 84%) as a yellow foam.

LCMS (Method A): R_T = 8.47 minutes; 334 $(M+H)^+$.

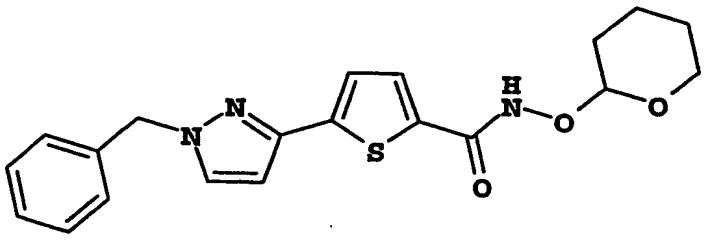
(j) 5-(1*H*-Pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using 5-(1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid [Reference Example 2(c)] there was prepared 5-(1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (66mg, 55%) as a foam.

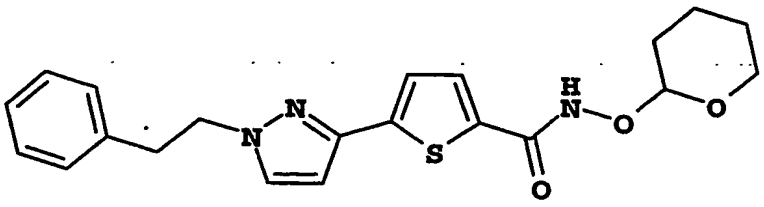
LCMS (Method A): R_T = 5.52 minutes; 294 $(M+H)^+$.

(k) 5-(1-Benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



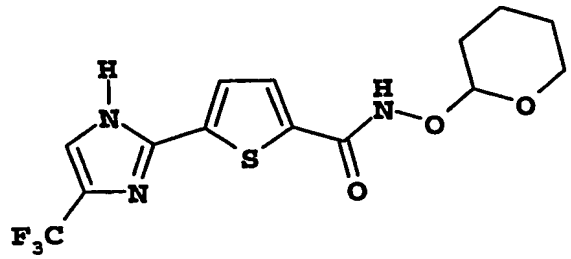
By proceeding in a similar manner to Reference Example 1(a) but using 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid [Reference Example 2(d)] there was prepared 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (65mg, 91%) as a colourless oil. LCMS (Method A): R_T = 8.39 minutes; 384 $(M+H)^+$.

(l) 5-(1-Phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



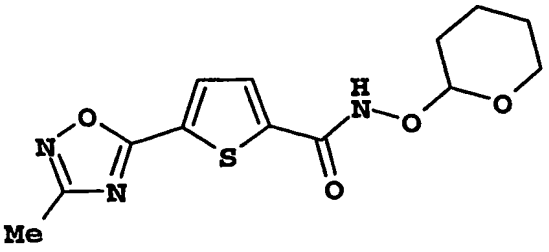
By proceeding in a similar manner to Reference Example 1(a) but using 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid [Reference Example 2(e)] there was prepared 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (138mg, 92%) as a colourless oil. LCMS (Method A): R_T = 8.79 minutes; 398 $(M+H)^+$.

(m) 5-(4-Trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



By proceeding in a similar manner to Reference Example 1(a) but using 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid [Reference Example 6] there was prepared 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (42mg, 80%) as a colourless gum. LCMS (Method A): R_T = 6.77 minutes; 362 $(M+H)^+$.

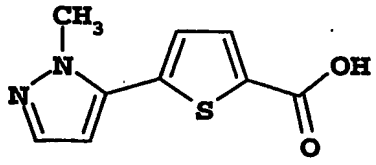
(n) 5-(3-Methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide



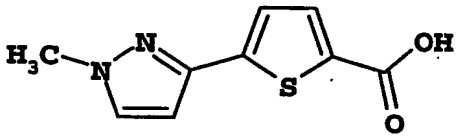
By proceeding in a similar manner to Reference Example 1(a) but using 5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid there was prepared 5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid (tetrahydro-pyran-2-yloxy)-amide (155mg, 98%) as colourless gum, which was used directly without further purification.

REFERENCE EXAMPLE 2

(a) 5-(2-Methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid and 5-(1-Methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid

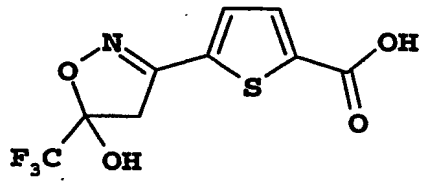


and



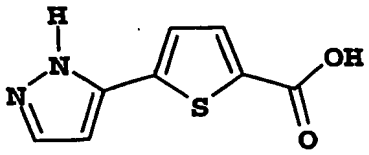
A mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carbonitrile and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile [0.7g, 3.7mmol, Reference Example 3(a)]) in sodium hydroxide solution (15ml, 1M) was heated at reflux for 2 hours. The reaction mixture was cooled to room temperature, diluted with water, acidified with hydrochloric acid (1M) and extracted three times with ethyl acetate. The combined extracts were dried over magnesium sulphate and then evaporated under reduced pressure. The residue was subjected to flash column chromatography to give a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (94mg, 12%) as a yellow solid. LCMS (Method B): R_T = 1.48 minutes; 209 $(M+H)^+$.

(b) 5-(5-Hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carboxylic acid



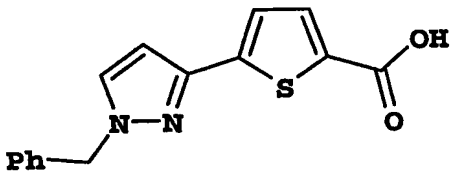
By proceeding in a similar manner to Reference Example 2(a) but using 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carbonitrile [Reference Example 7] there was prepared 5-(5-hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carboxylic acid (85mg, 74%) as a white solid. LCMS (Method A): R_T = 6.34 minutes; 282 $(M+H)^+$.

(c) 5-(1*H*-Pyrazol-3-yl)-thiophene-2-carboxylic acid



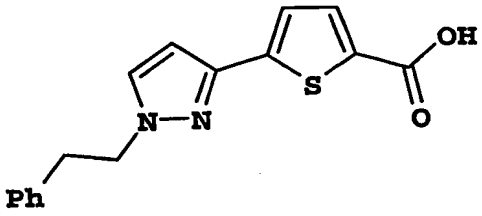
By proceeding in a similar manner to Reference Example 2(a) but using 5-(1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile [Reference Example 3(b)] there was prepared 5-(1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (97mg, 97%) as a yellow solid. LCMS (Method A): R_T = 4.79 minutes; 195 $(M+H)^+$.

(d) 5-(1-Benzyl-1*H*-Pyrazol-3-yl)-thiophene-2-carboxylic acid



By proceeding in a similar manner to Reference Example 2(a) but using 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile [Reference Example 8(a)] there was prepared 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (59mg, 96%) as a white powder. LCMS (Method A): R_T = 7.98 minutes; 285 $(M+H)^+$.

(e) 5-(1-Phenethyl-1*H*-Pyrazol-3-yl)-thiophene-2-carboxylic acid

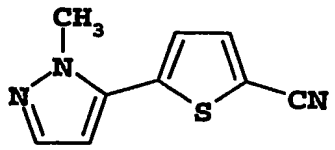


By proceeding in a similar manner to Reference Example 2(a) but using 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile [Reference Example 8(b)] there was prepared 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (116mg, 97%) as white solid.

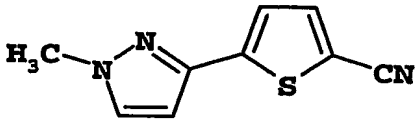
LCMS (Method A): R_T = 8.44 minutes; 299 $(M+H)^+$.

REFERENCE EXAMPLE 3

(a) 5-(2-Methyl-2*H*-pyrazol-3-yl)-thiophene-2-carbonitrile and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile

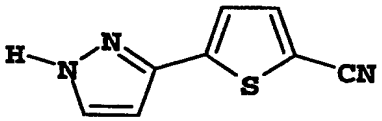


and



A solution of 5-(3-dimethylamino-acryloyl)-thiophene-2-carbonitrile [0.70g, 3.34mmol, Reference Example 4(a)]) in ethanol (30ml) was treated with methylhydrazine (0.19ml, 3.58mmol). The mixture was heated to reflux for 7 hours then cooled to room temperature and then concentrated under reduced pressure to give a mixture of 5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carbonitrile and 5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile (0.50g) which was used directly in the next step.

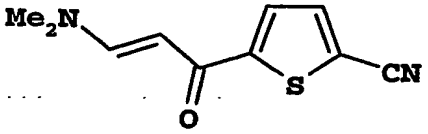
(b) 5-(1*H*-Pyrazol-3-yl)-thiophene-2-carbonitrile



By proceeding in a similar manner to Reference Example 3(a) but using 1.19g of 5-(3-dimethylamino-acryloyl)-thiophene-2-carbonitrile, 20ml of ethanol and hydrazine hydrate (0.20ml, 6.4mmol), heating the reaction mixture at reflux for 16 hours and partitioning the reaction product between ethyl acetate and water there was prepared 5-(1*H*-Pyrazol-3-yl)-thiophene-2-carbonitrile (0.80g, 89%) as a brown solid. LCMS (Method A): R_T = 5.90 minutes; 176 $(M+H)^+$.

REFERENCE EXAMPLE 4

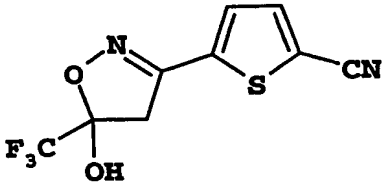
(a) 5-(3-Dimethylamino-acryloyl)-thiophene-2-carbonitrile



A solution of 5-acetylthiophene-2-carbonitrile (1.0g, 6.6mmol) in dimethylformamide (50ml) was treated with *tert*-butoxybis(dimethylamino)methane (1.7ml, 8.27mmol). The resulting yellow solution was heated at 70°C for 8 hours, then allowed to cool to room

temperature and then concentrated under reduced pressure. The residue was triturated with diisopropyl ether, concentrated to about 5ml and triturated again with pentane to give 5-(3-dimethylamino-acryloyl)-thiophene-2-carbonitrile (1.3g, 95%) as a yellow solid. LCMS (Method A): R_T = 5.63 minutes; 207 $(M+H)^+$.

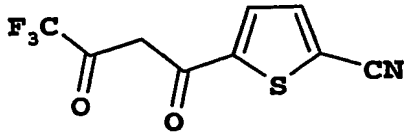
(b) 5-(5-Hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carbonitrile



A solution of 5-(4,4,4-Trifluoro-3-oxo-butyryl)-thiophene-2-carbonitrile (200mg, 0.81mmol [Reference example 5]) in ethanol (4ml) was treated with hydroxylamine hydrochloride (56mg, 0.81mmol) and acetic acid (4ml). The resulting solution was heated to reflux for 2 hours at which time t.l.c. analysis [ethyl acetate/petroleum ether fraction (b.p. 40-60°C) 7:3, v/v] indicated complete disappearance of the starting material. The mixture was allowed to cool to room temperature and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed with saturated sodium bicarbonate solution and then concentrated in vacuo. The residue was subjected to column chromatography on silica eluting with 10% - 19%(v/v) ethyl acetate in petroleum ether (bp 40-60°C) to give 5-(5-hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carbonitrile (162mg, 82%) as an off-white solid. LCMS (Method A): R_T = 7.63 minutes; 263 $(M+H)^+$.

REFERENCE EXAMPLE 5

5-(4,4,4-Trifluoro-3-oxo-butyryl)-thiophene-2-carbonitrile

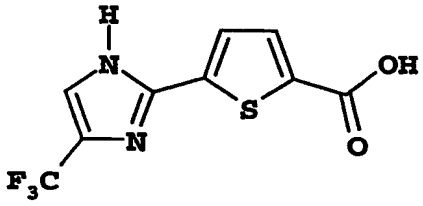


A suspension of sodium methoxide (384mg, 17.3mmol) in anhydrous diethyl ether (50ml) under nitrogen was treated with ethyl trifluoroacetate (1.97ml, 16.5mmol) followed by 5-acetylthiophene-2-carbonitrile (2.5g, 16.5mmol). The solution was stirred vigorously for

4 days and then quenched by the addition of hydrochloric acid (1M). The reaction mixture was extracted with ethyl acetate and the organic phase was washed with brine, then dried over sodium sulphate and then evaporated to give 5-(4,4,4-trifluoro-3-oxo-butyryl)-thiophene-2-carbonitrile (4.07g, 75%) as a brown solid, which was used without further purification. LCMS (Method C): R_T = 2.68 minutes; (-ve ion) 246 (M^-)

REFERENCE EXAMPLE 6

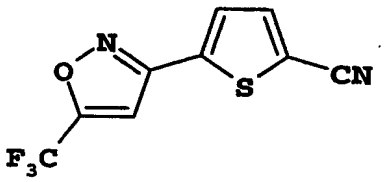
5-(4-Trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid



A suspension of 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid methyl ester (142mg, 0.51mmol, Reference Example 9) in a mixture of sodium hydroxide solution (15ml, 2M) and ethanol (15ml) was heated to 50°C for 15 minutes. The reaction mixture was allowed to cool to room temperature and then extracted five times with ethyl acetate. The combined extracts were dried over magnesium sulphate and then concentrated in vacuo to yield 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid (115mg, 85%) as a pale yellow powder. LCMS (Method A): R_T = 6.04 minutes; 263 $(M+H)^+$.

REFERENCE EXAMPLE 7

5-(5-Trifluoromethyl-isoxazol-3-yl)-thiophene-2-carbonitrile

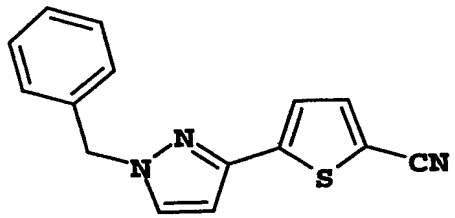


A solution of 5-(5-hydroxy-5-trifluoromethyl-4,5-dihydro-isoxazol-3-yl)-thiophene-2-carbonitrile [168mg, 0.64mmol, Reference example 4(b)]) in anhydrous dichloromethane (10ml), under nitrogen, was treated with molecular sieves and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.1ml, 0.67mmol). The mixture was refluxed for 2 hours and then the dichloromethane was evaporated and the residue was resuspended in

dichloroethane. The mixture was refluxed for 2.5 days and then filtered. The filtrate was concentrated *in vacuo* and the residue was subjected to flash column chromatography on silica eluting with 10% - 30% (v/v) ethyl acetate in petroleum ether fraction (b.p. 40-60°C) to yield 5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carbonitrile (136mg, 87%) as a white solid. LCMS (Method A): R_T = 9.83 minutes; 337.

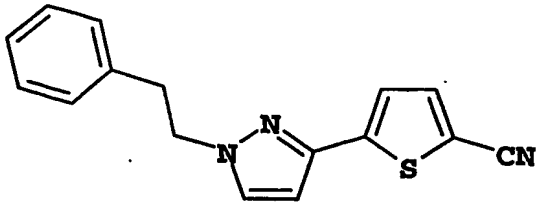
REFERENCE EXAMPLE 8

(a) 5-(1-Benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile



A solution of 5-(1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile [98mg, 0.55mmol, Reference Example 3(b)]) in toluene (6ml) was treated with potassium hydroxide (25mg, 0.44mmol), potassium carbonate (61mg, 0.44mmol), tetrabutylammonium hydrogen sulphate (23mg, 0.066mmol) and benzyl chloride (76µl, 0.66mmol). The mixture was refluxed overnight after which t.l.c. (ethyl acetate 3:2 petroleum ether, bp 40-60°C) indicated the presence of remaining starting material. A further aliquot of benzyl chloride (76µl, 0.66mmol) was added and the mixture was refluxed for a further 40 hours. The reaction mixture was filtered and the residue was washed with toluene. The combined filtrate and washings were concentrated *in vacuo* and the residue was partitioned between ethyl acetate and brine. The two phases were separated and the organic phase was dried over sodium sulphate and then concentrated *in vacuo*. The crude product was subjected to column chromatography on silica eluting with 8% (v/v) ethyl acetate in petroleum ether fraction (b.p. 40-60°C) to yield 5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile (63mg, 43%) as a yellow powder. LCMS (Method A): R_T = 9.72 minutes; 266 $(M+H)^+$.

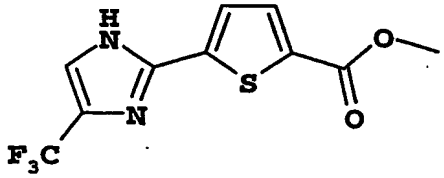
(b) 5-(1-Phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile



By proceeding in a similar manner to Reference Example 8(a) but using 2-bromoethyl benzene and subjecting the reaction product to column chromatography on silica eluting with 7.5% - 12% (v/v) ethyl acetate in petroleum ether fraction (b.p. 40-60°C) there was prepared 5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carbonitrile (118mg, 89%) as a white solid. LCMS (Method A): R_T = 10.14 minutes; 280 $(M+H)^+$.

REFERENCE EXAMPLE 9

5-(4-Trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid methyl ester



To a solution of sodium acetate (1.2g, 13.9mmol) in water (15ml) was added 1,1-dibromo-3,3,3-trifluoroacetone (0.80ml, 5.37mmol) and the resulting mixture was heated to 80°C for 45 minutes. The solution was cooled to 0°C and 5-formyl-thiophene-2-carboxylic acid methyl ester (0.84g, 4.92mmol) in methanol (20ml) was added followed by conc. ammonium hydroxide solution (25ml) and the solution was allowed to warm to room temperature overnight. The reaction mixture was concentrated and the aqueous residue was extracted three times with ethyl acetate. The combined organic phase was evaporated and the crude product was purified by column chromatography on silica eluting with 10% v/v ethyl acetate in dichloromethane to yield 5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid methyl ester (0.22g, 16%) as a pale yellow powder. LCMS (Method A): R_T = 7.55 minutes; 277 $(M+H)^+$.

Biological Activity

Compounds were tested for their capacity to inhibit histone deacetylase activity (primary assay) and for their biological effects on growing cells (secondary assay).

Deacetylase Assay

Total lysates from K562 chronic human myelogenous leukemia cells (obtained from American Type Culture Collection, Rockville, MD) are used as source of HDAC activity. Cells are grown in RPMI media supplied with 10% FCS, harvested by centrifugation, washed once in PBS and resuspended at a density of $24x10^6$/ml in HDA buffer (15mM Potassium phosphate pH 7.5, 5% glycerol, 0.2mM EDTA). After sonication, lysates are centrifuged at 1000g for 20 minutes and the resulting supernatant is aliquoted and stored at –80°C. Alternatively, commercially available HeLa nuclear extracts (BIOMOL) are used as source of histone deacetylase activity.

The assay was carried out using the commercially available "HDAC Fluorescent Activity Assay" (BIOMOL) according to the manufacturer instructions. When deacetylation of the lysine occurs, the substrate can react with the added developer producing a fluorophore. The amount of fluorophore produced is proportional to the HDAC activity in the sample and is quantified using a multiwell fluorimeter capable of excitation at 360nm and detection at 450nm.

Compounds are diluted in DMSO prior to addition to assay buffer, the final DMSO concentration in the assay being 1%.

The percent activity of the compounds in reducing histone deacetylase enzymatic activity is calculated as follows:

$$\% \text{ activity} = \{ (F^S - B) / (F^C - B) \} \times 100$$

where:

F^S is the fluorescence at 450nm in the presence of the tested compound (Sample).

F^C is the fluorescence at 450nm in the presence of vehicle 1 % DMSO (Control).

B is the fluorescence at 450nm in the absence of enzyme (Background fluorescence)

The IC_{50} is defined as the concentration at which a given compound achieves 50% activity. IC_{50} values are calculated using the XLfit software package (version 2.0.5).

Table 1 shows the results obtained for the compounds of the present invention.

Table 1

Sample	IC_{50} / μM
Example 1 (a)	0.20
Example 1 (g)	0.42
Example 1 (l)	0.06

Secondary Assay

Compounds are tested in a cell proliferation assay using the following cell lines:

MCF-7 human mammary gland adenocarcinoma (ATCC)
MDA-MB-231 human mammary gland adenocarcinoma (ATCC)

Both cell lines are free of *Mycoplasma* contamination (PCR Mycoplasma Detection Set, Takara). MCF-7 are kept in MEM medium (Gibco) supplemented with 10% FCS and 1% Non Essential Amino Acids at 37°C in a 5% CO_2 humidified incubator.
MDA-MB-231 are kept in L-15 (Leibovitz) medium (Gibco) supplemented with 15% FCS at 37°C in a non-modified atmosphere, humidified incubator.

Cells are seeded in 96-well plates at a density of 20,000 cells/ml (3,000 cells/well) and after 24h they are exposed to different concentrations of compounds in 0.1% DMSO. Cells are grown for a further 72h, fixed in 0.5% glutaraldehyde and stained with 0.25% Crystal Violet. This is a dye that binds to chromatin and, after extensive washes with H_2O, can be solubilised in 10% Acetic Acid. The solubilised Crystal Violet is proportional to the

number of cells present in each well and can be quantified using a multiwell spectrophotometer by measuring the absorbance of the solution at 595nm.

The percent activity of the compounds in reducing cell number is calculated as follow:

$$\% \text{ activity} = \{ (A^S - B) / (A^C - B) \} \times 100$$

where:

A^S is the absorbance at 595nm in the presence of the tested compound (Sample).
A^C is the absorbance at 595nm in the presence of vehicle 0.1% DMSO (Control).
B is the absorbance at 595nm in the absence of cells (Background staining).

The IC_{50} is defined as the concentration at which a given compound achieves 50% activity. IC_{50} values are calculated using the XLfit software package (version 2.0.5).

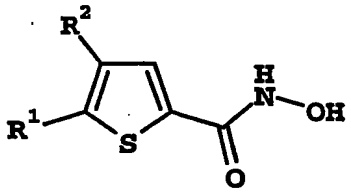
Table 2 shows the results obtained for the compounds of the present invention.

Table 2

Sample	**MCF-7 IC_{50} / μM**	**MDA-MB-231 IC_{50} / μM**
Example 1 (a)	11	32
Example 1 (g)	31	44
Example 1 (l)	2.1	4.5

CLAIMS

1. A compound of formula (I):



(I)

in which

R^1 represents aryl or heteroaryl, each optionally substituted by one or more groups selected from R^3, alkylenedioxy, carboxy, cyano, halo, hydroxy, nitro, haloalkyl, haloalkoxy, $-C(=O)-R^3$, $-C(=O)-OR^3$, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=O)-OR^3$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-C(=Z)-R^3$, $-O-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^3$, $-OR^3$, $-O-C(=O)R^3$, $-SH$, $-SR^3$, $-SOR^3$, $-SO_2R^3$ and $-SO_2-NR^4R^5$;

R^2 represents hydrogen, chloro, cyano, fluoro, alkoxy, alkyl, or haloalkyl;

R^3 represents aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl or R^m;

R^4 and R^5 independently represent a group selected from hydrogen, alkyl, alkenyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl, wherein said alkyl or alkenyl are optionally substituted by aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl; or the group $-NR^4R^5$ may form a cyclic amine;

R^6 represents hydrogen or lower alkyl;

R^m represents alkyl, alkenyl and alkynyl, wherein said alkyl, alkenyl or alkynyl are optionally substituted by one or more groups selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, hydroxy, $-C(=Z)-NR^4R^5$, $-NR^4R^5$, $-NR^6-C(=Z)-R^n$, $-O-C(=O)-NR^4R^5$, $-NR^6-C(=O)-OR^n$, $-NR^6-C(=O)-NR^4R^5$, $-NR^6-SO_2-R^n$, $-OR^n$, $-SOR^n$, SO_2R^n and $-SO_2-NR^4R^5$;

R^n represents alkyl, alkenyl or alkynyl, optionally substituted by one or more groups selected from aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, hydroxy and halogen; or R^n represents aryl, heteroaryl, cycloalkyl, cycloalkenyl or heterocycloalkyl; and

Z is O or S,

and corresponding *N*-oxides, pharmaceutically acceptable salts, solvates and prodrugs of such compounds.

2. A compound according to claim 1 wherein R^1 is optionally substituted phenyl.

3. A compound according to claim 1 or 2 wherein R^1 is 4-methoxyphenyl.

4. A compound according to claim 1 wherein R^1 is selected from optionally substituted monocyclic heteroaryl.

5. A compound according to claim 4 wherein R^1 is selected from optionally substituted imidazolyl, isoxazolyl, oxadiazolyl, pyrazolyl, pyridinyl, thienyl and pyrimidinyl.

6. A compound according to claim 5 wherein R^1 is selected from 1-(2-phenylethyl)-1*H*-pyrazol-3-yl, 1-benzyl-1*H*-pyrazol-3-yl, 4-trifluoromethyl-1*H*-imidazol-2-yl, pyridin-2-yl, 5-trifluoromethyl-1*H*-pyrazol-3-yl, 1-methyl-1*H*-pyrazol-3-yl, 2-methyl-2*H*-pyrazol-3-yl, 1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl, 2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl, 1*H*-pyrazol-3-yl, pyridin-4-yl, 5-trifluoromethyl-isoxazol-3-yl, 3-methyl[1,2,4]oxadiazol-5-yl, or thiophene-2-yl.

7. A compound according to any preceding claim wherein R^2 is hydrogen.

8. A compound according to any preceding claim wherein R^3 is methyl.

9. A compound according to any preceding claim wherein R^4 and R^5 are independently selected from hydrogen and alkyl.

10. A compound according to claim 1 selected from :
5-(2-methyl-5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(2-methyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(5-trifluoromethyl-2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(1-methyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(5-trifluoromethyl-isoxazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-phenyl-thiophene-2-carboxylic acid hydroxyamide;
5-pyridin-2-yl-thiophene-2-carboxylic acid hydroxyamide;
[2,2']bithiophenyl-5-carboxylic acid hydroxyamide;
5-(4-methoxy-phenyl)-thiophene-2-carboxylic acid hydroxyamide;
5-(2*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(1-benzyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(1-phenethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid hydroxyamide;
5-(4-trifluoromethyl-1*H*-imidazol-2-yl)-thiophene-2-carboxylic acid hydroxyamide; and
5-(3-methyl-[1,2,4]oxadiazol-5-yl)-thiophene-2-carboxylic acid hydroxyamide;
and *N*-oxides, pharmaceutically acceptable salts, solvates and prodrugs of such compounds.

11. A compound according to any of claims 1 to 10 , for use in therapy.

12. The use of a compound according to any of claims 1 to 10 in the manufacture of a medicament for the treatment of a disease in which inhibition of histone deacetylase can prevent, inhibit or ameliorate the pathology and/or symptomatology of the disease.

13. A method for treating a disease in a patient in which inhibition of histone deacetylase can prevent, inhibit or ameliorate the pathology and/or symptomatology of the disease, which method comprises administering to the

patient a therapeutically effective amount of a compound according to any of claims 1 to 10.

14. A method or use according to claim 12 or 13 wherein said disease is a disease caused by increased cell proliferation.

15. A method or use according to claim 12 or 13 wherein said disease is cancer, psoriasis, fibroproliferative disorders, smooth muscle cell proliferation disorders, inflammatory diseases and conditions treatable by immune modulation, neurodegenerative disorders, diseases involving angiogenesis, fungal and parasitic infections and haematopoietic disorders.

16. A method or use according to claim 12 or 13 wherein said disease is liver fibrosis, arteriosclerosis, restenosis, rheumatoid arthritis, autoimmune diabetes, lupus, allergies, Huntington's disease, retinal diseases, protozoal infections, anaemia, sickle cell anaemia and thalassemia.

17. A method or use according to claim 16 wherein said protozoal infection is malaria, toxoplasmosis or coccidiosis.

18. A method or use according to claim 16 wherein said retinal disease is diabetic retinopathy, age-related macular degeneration, interstitial keratitis or rubeotic glaucoma.

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